SPARTINA SPECIES ZONATION ALONG AN ESTUARINE GRADIENT IN GEORGIA: EXPLORING MECHANISMS CONTROLLING DISTRIBUTION

by

SUSAN N. WHITE

(Under the Direction of Merryl Alber)

ABSTRACT

The general paradigm for the observed plant zonation in salt marshes is that a combination of abiotic stress and competition drive vegetation patterns within a *single* marsh, but there have been few studies investigating the bankside vegetation changes that occur along the longitudinal salinity gradient of estuaries. The main objectives of the research presented in this dissertation were to examine whether the same controls that explain the distribution of *Spartina alterniflora* in the salt marsh can be applied without modification to a longitudinal salinity gradient and to evaluate changes in *Spartina* distributions under drought conditions. Reciprocal transplant studies, greenhouse experiments, species removals in mixed *Spartina* stands, and vegetation surveys were conducted in the estuary of the Altamaha River, GA, where *S. cynosusroides* occurs upstream of *S. alterniflora*. In reciprocal transplant experiments, each plant survived and performed best in its natural habitat. The presence of conspecific neighbors slightly reduced *S. alterniflora* plant performance in the salt marsh whereas *S. cynosuroides* showed little response in either environment. The results of these and other experiments suggest the lower estuarine distribution of *Spartina cynosuroides* is controlled by abiotic

conditions (salinity or sulfide concentrations). The upper estuarine distribution of *S. alterniflora* is not well understood, but seems to also be primarily controlled by abiotic factors (possibly a sulfate requirement). These outcomes challenge results from previous investigations of zonation controls in salt marshes and suggest that modifications to the salt marsh paradigm are necessary when describing vegetation distribution along an estuarine gradient. During an extended drought (2000-2002), *Spartina alterniflora* density increased to a greater extent than *S. cynosuroides* in mixed stands and the location where *Spartina* cover was 50 % *S. cynosuroides* and 50 % *S. alterniflora* shifted approximately 3 km upriver, suggesting that *Spartina* communities can respond rapidly to increasing estuarine salinity. These studies improve our understanding of the ecological linkages in estuaries and can aid coastal policymakers in making better management decisions and predictions concerning how changes in freshwater inflow might impact the distribution of estuarine organisms.

INDEX WORDS: *Spartina alterniflora*, *Spartina cynosuroides*, Plant zonation, Freshwater inflow, Salt marsh, Brackish marsh, Altamaha River estuary, Georgia coast

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DEDICATION

For their unwavering encouragement, realistic perspectives, and endless love, I dedicate this dissertation to my parents, Richard and Norma White, my grandmother, Katharine White, and my sister and brother, Karen and Richard White.

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CHAPTER 1

INTRODUCTION

OVERVIEW

The intertidal marshes that border riverine estuaries grade from freshwater marshes (where salinities are <0.5 practical salinity units, or psu), to brackish marshes (with salinities between 0.5-18 psu), to salt marshes (with salinities >18 psu), each of which has a characteristic vegetation pattern. The factors that control plant distribution patterns in salt marshes have received the most attention, and the generally accepted paradigm is that a combination of abiotic stress (specifically salinity) and competition drive vegetation patterns within a *single* marsh. However, there have been few studies investigating the vegetation changes that occur along the longitudinal salinity gradient of estuaries and it is unclear if the same mechanisms that control the distribution of plants in salt marshes can be applied.

In the southeastern United States, *Spartina cynosuroides* dominates riverbank habitats in brackish areas, whereas *S. alterniflora* is dominant in salt marshes. The central goal of this dissertation was to investigate the mechanisms that control the distribution of *Spartina* species along the longitudinal axis of an estuary. Specific research objectives were to document the distribution of *S. alterniflora* and *S. cynosuroides* in response to changing inflow conditions and to determine the effects of both abiotic (salinity and sulfate concentrations) and biotic (neighbor presence) factors on plant performance. This type of study is important for improving our understanding of

the ecological linkages in estuaries and will also help us to predict how changes in estuarine salinity regimes, mediated by changes in freshwater inflow, might affect the distributions of estuarine organisms.

SALT MARSH STUDIES

Spartina alterniflora is the dominant plant species in southeastern United States salt marshes. *Spartina alterniflora* is tallest on the creekbank and plant height decreases with distance from the creekbank to upland areas where mixed marsh communities of *S. alterniflora* and *Juncus romereianus* are located. Early work focusing on plant community structure in salt marshes identified various abiotic mechanisms that structure these habitats, including plant response to salinity, inundation (resulting in anoxic sediments), and nutrient availability (Valiela and Teal 1974, Mendelssohn 1979, King et al. 1982, Howes et al. 1986, Pennings and Bertness 2001). More recent research has focused on combinations of the above abiotic mechanisms with biotic interactions (such as competition or facilitation) in order to more fully explain observed plant distributions in the field (Bertness and Ellison 1987, Bertness and Yeh 1994, Hacker and Gaines 1997, Gough and Grace 1998, Pennings and Bertness 2001). In the salt marsh, both inter- and intra-specific competition for light, nutrients and water influence plant community structure. However, competitive abilities are influenced by an organism's physiological ability to tolerate environmental stressors. Several studies have found that competitively dominant organisms monopolize physically benign habitats while the less competitive (but often more physiologically hardy) organisms dominate physically stressful habitats (Connell and Slayter 1977, Adam 1990, Bertness and Pennings 2000).

The most important abiotic factors that control zonation in New England salt marshes is different plant tolerances to environmental stresses such as flood duration and wrack disturbance (Mendelssohn 1979, Bertness and Ellison 1987, Bertness 1991). In contrast, studies in low-latitude salt marshes indicate that salinity stress is an important abiotic factor (Pennings and Bertness 2001). This is due to the greater solar radiation (and, hence, greater evapotranspiration) experienced at lower latitudes, which results in elevated salinities in high marsh areas where tidal flushing and freshwater availability is minimal. This harsh physical environment limits the expansion of competitively inferior plants into high marsh habitat (Mahall and Park 1976, Wiegert et al. 1983, Zedler and Beare 1986, Pennings and Bertness 2001). Thus, physiological tolerance to salinity is recognized as one of the main mechanisms defining plant zonation in low-latitude coastal salt marshes (Odum 1988, Mitsch and Gosselink 1993). However, biotic interactions (competition and facilitation) may also be important. Experimentally changing salinity levels in low-latitude marshes alters plant distributions as a result of the plant's response to both salinity stress *and* species interactions (McKee and Mendelssohn 1989, Brewer and Grace 1990, Howard and Mendelssohn 1999).

Nutrient availability can also influence marsh plant zonation. Plant communities can vary among areas that have different nutrient concentrations (Levine et al. 1998). Experimental additions of nitrogen in field manipulations can change plant community structure in the salt marsh, allowing the expansion of previously nutrient-limited plants (i.e. *S. alterniflora*, *Salicornia*) into new marsh habitat (Valiela and Teal 1974, Covin and Zedler 1988, Valiela 1995, Levine et al. 1998). Additional studies, however, that quantified available inorganic nitrogen concentrations in the marsh documented that

interstitial ammonium (the dominant form of inorganic nitrogen in most salt marshes) was often present at a larger concentration in the less productive, inland areas where short *S. alterniflora* plants are found, than in the more productive streamside areas with much taller *S. alterniflora* stands (Mendelssohn 1979, Craft et al. 1991). This presented an interesting paradox: why were the short *Spartina* plants not utilizing the available nitrogen?

Concurrent with the investigation of available pools of nitrogen in marsh soils was the observation that porewater exchange in streamside marsh zones was greater than that found in inland areas (Mendelssohn and Seneca 1980, Howes et al. 1981). The difference in water exchange between these two areas results in a difference in the amount of available oxygen (or soil redox potential, Eh) within the soil. Specifically, inland soils are more reduced while the streamside soils are more oxidized (Mendelssohn and Seneca 1980, Howes et al. 1981). Mendelssohn (1981) indicated that the aerenchyma tissue (tissue that conducts oxygen to roots) in *S. alterniflora* plants located in highly reduced, waterlogged soils did not provide sufficient oxygen to support aerobic respiration in the roots. The resulting anaerobic metabolism produced less energy for plant use (in nutrient uptake and growth processes) and thus resulted in reduced plant growth (Mendelssohn et al. 1981). Correlations between redox potential and/or pH in the field have been linked to reductions in nutrient availability and reductions in *S. alterniflora* production (Linthurst 1979, Mendelssohn et al. 1981).

In addition to the reducing conditions (lower Eh), higher dissolved sulfide concentrations are found at inland sites in comparison to streamside areas and this may also play a role in plant zonation (King et al. 1982, DeLaune et al. 1983, Mendelssohn

and McKee 1988). King et al. (1982) identified an in-situ sulfide concentration range of 0.09-3.0 mM in marsh soils (between 0 and 30 cm in depth) and found an inverse relationship between sulfide concentration and biomass of *S. alterniflora* in marshes of Sapelo Island, Ga. Similar observations were made in Louisiana marshes by DeLaune et al. (1983). Bradley and Dunn (1989) conducted a hydroponic culture experiment to investigate the growth response of *S. alterniflora* and *S. cynosuroides* to varying sulfide concentrations. They found that *S. alterniflora* production was inhibited at sulfide concentrations as low as 1.0 mM, suggesting a role for sulfide in constraining marsh production. A similar reduction in *S. alterniflora* biomass production was noted in a greenhouse sulfide addition experiment (Koch and Mendelssohn 1989). *Spartina cynosuroides* was inhibited at sulfide concentrations greater than 0.5 mM, suggesting that this species may be less tolerant to high sulfide concentrations (Bradley and Dunn 1989).

The mechanisms by which hydrogen sulfide acts to inhibit plant growth may be related to reductions in nitrogen uptake. Hydrogen sulfide accumulates in porewater as a result of the biologically mediated reduction of sulfate to sulfide in anoxic environments by SO_4^{-2}–reducing bacteria (e.g. *Desulfovibrio*) that use sulfate as the terminal electron acceptor for carbon oxidation (Postgate 1979, Koch et al. 1990). Sulfate reduction is the dominant anaerobic heterotrophic process in salt marshes and degrades approximately twelve times more organic matter than oxygen respiration and denitrification combined (Howarth and Teal 1980, Howarth and Hobbie 1982). Hydrogen sulfide is a known phytotoxin that can inhibit plant growth and nitrogen uptake kinetics at high concentrations by decreasing the ability of the plant to generate sufficient energy anaerobically (via fermentation) (King et al. 1982, Ingold and Havill 1984, Bradley and

Morris 1990, Koch et al. 1990). Koch et al. (1990) experimentally added sulfide to *S. alterniflora* and monitored root energy status as well as metabolic pathways. They observed a decrease in nitrogen uptake as well as a decrease in leaf elongation, providing direct evidence of sulfide-induced inhibition of nitrogen uptake and plant growth in *S. alterniflora*. The presence of sulfide can inhibit alternate anaerobic metabolic pathways (via inhibition of alcohol dehydrogenase, ADH), thus limiting important energy-dependent functions such as nutrient uptake (Koch et al. 1990). Additional laboratory culture experiments by Bradley and Morris (1990) support the assertion that nitrogen uptake is hindered at the high sulfide concentration found in areas of low redox potential. An experiment by Wiegert et al. (1983) showed that with increased water exchange, interstitial sulfide concentrations decreased and there were concurrent increases in *S. alterniflora* biomass.

Interestingly, although most of the above research focuses on the negative effects of high porewater sulfide concentrations (>1.0 mM) on the growth of both *Spartina* species, lower concentrations of sulfide (0-1.0 mM) appear to stimulate *S. alterniflora* growth (Bradley and Dunn 1989, Morris et al. 1996). This stimulation may be due to secondary nutrient effects (sulfide presence increases solubility of specific nutrients) (Lambers et al. 1998) or it may be an energy subsidy provided by sulfide itself (Mendelssohn and Morris 2000).

BRACKISH MARSH STUDIES

In brackish marshes, *S. cynosuroides* dominates riverbank habitats, whereas *J. roemerianus* is found at intermediate and higher elevations. There are fewer studies that investigate the mechanisms that control plant distributions in the brackish marsh as

compared to the salt marsh. Generally, *S. alterniflora* is not found along creekbanks in brackish environments and Stribling (1994) observed that this species' upriver distribution may be limited by a sulfate requirement. Sulfate limitation obviously is not an issue in polyhaline marshes where seawater sulfate supplies are high (~28 mM; Pilson 1998). However, sulfate concentrations in oligohaline marshes are variable (Stribling 1994) as a result of both the irregularity in salinity of estuarine waters as well as the sulfur cycling in the marsh sediments. It is possible that, with the combination of low concentrations of sulfate in flooding waters in the brackish marsh, and high rates of sulfate reduction in marsh soils, the rhizosphere, or root zone, of the marsh plants will be depleted of sulfate (Wiebe et al. 1981, Stribling 1994). Stribling (1997) investigated the impacts of varying sulfate concentrations on the growth of *S. alterniflora* and *S. cynosuroides* in a controlled greenhouse experiment. She documented a negative response of *S. alterniflora* to low sulfate concentrations (<0.5 mM) and suggested that this plant's adaptation to high salinity is linked to a high sulfate requirement. The optimal sulfate range for *S. alterniflora* in these experiments (between 0.5-1.0 mM) was representative of the concentrations found in estuarine waters of approximately 1 psu. This was consistent with field observations that *S. alterniflora* was found in both polyhaline marshes as well as oligohaline marshes where salinities were around 2 psu (Stribling 1994). Additional support for the idea that *S. alterniflora* requires unusually high amounts of sulfate is found when one compares the nitrogen/sulfur ratio observed in *S. alterniflora* (3:1) (Ornes and Kaplan 1989, Stribling 1994) to the ratio of a typical plant (not deficient in sulfur) of 20:1 (Tabatabai 1984, Cram 1990). These results suggest that sulfate availability may influence the upstream extent of *S. alterniflora*.

OBJECTIVES

The broad objective of this research was to investigate changes in the distribution, diversity and interactions of coastal brackish and salt marsh vegetation along a salinity gradient in the Altamaha River estuary, GA. The Altamaha River watershed in Georgia is one of the largest on the East Coast with an area over 37,300 km^2 (Dame et al. 2000). Typically there is substantial freshwater discharge into the estuary and salt water is generally unable to penetrate far upstream. However, between 1999-2002 inflows of this river decreased considerably due to prolonged drought conditions. Median discharge for the Altamaha River from 1968-1997 was 250 m^3 s^{-1} (Alber and Sheldon 1999) whereas the median discharge for the drought years was 124 m^3 s^{-1} (J. Sheldon, pers. com). Not unexpectedly, salinities increased further upstream, due to tidal salinity intrusion, such that salinities as great as ~10 psu were recorded 16 km from the mouth of the Altamaha (in 2001) and sustained average salinities ~ 3 psu were observed 20 km upriver (in 2000 and 2001) (Georgia Coastal Long Term Ecological Research Monitoring Data). This shift in salinity provided an opportunity to assess the response of both *S. alterniflora* and *S. cynosuroides* to changing environmental conditions, and to evaluate the relative importance of biotic and abiotic controls of plant distribution.

The three primary chapters in this dissertation describe different experimental tests of *Spartina* species responses to varying conditions. Chapter 2 describes the results of a field reciprocal transplant study at salinity extremes along the Altamaha River estuary, GA. *Spartina alterniflora* and *S. cynosuroides* survival and performance were assessed with regard to abiotic (i.e. salinity and sulfate) and plant-plant interactions (i.e. competition and facilitation) in both transplant environments. These results are used to

explain whether abiotic and/or biotic mechanisms control the estuarine distributions of *S. cynosuroides* and *S. alterniflora*. Chapter 3 describes a series of greenhouse experiments that were conducted to investigate the growth response of both *S. alterniflora* and *S. cynosuroides* to field ranges of salinity and sulfate concentrations as well as to explicitly address the influence of neighbors on plant performance. Results from these experiments were used to help explain field distributions of these species. Finally, Chapter 4 describes two vegetation surveys that were conducted along the length of the Altamaha River, and a species removal experiment in mixed *Spartina* marshes that were used to assess changes in *Spartina* species distributions over the course of a drought period. The results from this effort were evaluated for their utility in the development of estuarine bioindicators that might be applied in conservation and management initiatives for freshwater inflow regulation.

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CHAPTER 2

EXTENDING THE SALT MARSH PARADIGM: CONTROLS OF *SPARTINA* DISTRIBUTIONS ALONG A GEORGIA ESTUARINE GRADIENT

INTRODUCTION

In contrast to the well studied phenomenon of plant zonation along the elevation gradient of salt marshes, very few studies have examined the control of plant zonation along the longitudinal gradient of an estuary, where there is a transition from tidal freshwater marsh upstream, to brackish marsh in intermediate areas, to salt marsh closest to the ocean. In the salt marshes of the southeastern United States, *Spartina alterniflora* dominates at lower elevations; *Juncus roemerianus* and *Distichlis spicata* are found in areas that are infrequently flooded at mid-elevation; and the upland border is frequently defined by the shrubs *Iva frutescens* and *Borrichea frutescens* (Wiegert and Freeman 1990). Brackish marshes in the southeast are comprised of a mix of *S. alterniflora* and *S. cynosuroides* at creekbank elevations with monospecific stands of *J. roemarianus* generally dominating at higher elevations (Wiegert and Freeman 1990, Higinbotham et al. 2004). Although the factors that control plant distribution along the salinity gradient of estuaries are not well understood, zonation patterns along the elevational gradients of salt marshes have been studied for decades.

The distribution of vegetation in salt marsh environments is strongly influenced by both abiotic and biotic factors. Tidal inundation in the salt marsh creates a strong salinity gradient from the creekbank inland, and numerous researchers have demonstrated

that plant physiological tolerance to salinity is one of the major mechanisms generating the zonation patterns observed in the field (Odum 1988, Mitsch and Gosselink 1993). Pennings and Callaway (1992) found that *Arthrocnemum subterminalis* (Parish's glasswort) was able to tolerate high salinities to a greater extent than *Salicornia virginica* (pickleweed), and its salt tolerance defined its distribution in saltier, higher elevations in a California marsh. Rozema et al. (1985) tested the salt and flooding tolerances of 12 plant species found in a Dutch marsh and concluded that their ranking in salt tolerance corresponded to their presence along the elevation of the marsh from the creekbank to upland areas. Bertness (1991a, 1991b, 1992) identified that the lower elevation, creekbank, distributions of *S. alterniflora* in a New England marsh are set by physical conditions, such as salinity, whereas the higher elevation distribution borders are typically set by competitive interactions. Generally, marsh plants that are more salt tolerant (e.g. *Spartina alterniflora*) are found closer to the creekbank than those that are less tolerant (e.g. *Iva frutescens*).

Additional abiotic factors such as oxygen and sulfide concentrations and soil drainage also influence salt marsh plant production and competitive ability. Low oxygen concentrations around the rhizosphere result from extensive water logging or from a reduction in belowground root biomass. These low oxygen environments negatively impact the ability of marsh plants to succeed in the environment and allow more physiologically hardy plants to dominate (Howes et al. 1981, Mendelssohn et al. 1981, Mendelssohn and McKee 1988). Mendelssohn and Seneca (1980) and Howes et al. (1981) observed that porewater exchange was greatest in creekbank zones where tall and medium height forms of *S. alterniflora* are located and decreased in inland zones where

short *Spartina* was observed. This difference in drainage influences soil redox potential, and results in more reduced sediment with higher sulfide concentrations at inland versus creekbank sites. With an increase in sulfide concentrations, a marsh plant's ability to access available nitrogen from the environment is inhibited and thus biomass production is constrained (King et al. 1982). Armstrong et al. (1985) found that the zonal distribution of vegetation in a salt marsh in Yorkshire, England, correlated with differences in the degree of soil aeration and differential tolerance of species to these environments. Differences in soil chemistry, as a result of variation in soil inundation and aeration, generate a gradient in production from the tall *Spartina* at the creekbank to the short *Spartina* in inland areas (King et al. 1982, DeLaune et al. 1983, Mendelssohn and McKee 1988).

In addition to a plant's response to the abiotic environment, the species distribution within a salt marsh can also be influenced by plant-plant interactions. Competitive interactions can limit the landward or upper-elevation distributions such that poorly competing species are competitively displaced to lower tidal elevations (Bertness 1991b, Pennings and Callaway 1992, Levine et al. 1998, Hacker and Bertness 1999a) whereas facilitation between species can buffer harsh environmental conditions (Bertness et al. 1992, Bertness and Shumway 1993, Bertness and Yeh 1994, Bertness and Hacker 1994, Hacker and Gaines 1997, Hacker and Bertness 1999b). For example, Bertness and Ellison (1987) found that *S. alterniflora* growth was enhanced when it was transplanted to the high marsh, where it is not normally found, if its competitors there were removed. Facilitiation, another type of plant-plant interaction, was demonstrated when bare marsh areas were initially colonized by *Distichlis* and *Spartina patens,* ameliorating harsh

salinity conditions by shading the substrate, thus allowing for the establishment and subsequent dominance of *Juncus* (Bertness and Shumway 1993).

The processes that generate the observable patterns of salt marsh vegetation distribution are often intricately linked such that multiple mechanisms (both abiotic and biotic), acting together within the complexity of the marsh system, more completely explain vegetation distribution (Callaway and Walker 1997). In a New England salt marsh, the distribution of the upland shrub, *Iva frutescens*, was influenced not only by the presence or absence of *Juncus gerardi* but was also constrained by its physiological tolerance to both salinity and waterlogging (Hacker and Bertness 1995). The removal of *J. gerardi* from mixed stands of *J. gerardi* and *I. frutescens* in a low elevation intertidal salt marsh decreased *I. frutescens* biomass, growth and survival suggesting a positive facilitative interaction between these two plants in this harsh environment. At higher intertidal elevations, however, where less stressful abiotic conditions exist, the interaction changed from facilitation to competition: *I. frutescens* outcompeted *J. gerardi*, thus creating a monoculture environment (Bertness and Hacker 1994). Levine et al. (1998) found that nutrient limitation, competition and physical stress all interact to structure salt marsh zonation in a New England marsh, whereas Pennings and Callaway (1992) found that flooding, soil salinity and competition interacted to determine plant zonation patterns in a California salt marsh. Kiehl et al. (1997) found that the addition of nitrogen to two Wadden-Sea salt marshes resulted in the replacement of *Puccinellia* by *Suaeda* as a result of competitive exclusion. Experimental manipulations of nutrient concentrations in salt marshes can reverse plant interactions and result in changes in the spatial arrangement of plants (Valiela and Teal 1974, Levine et al. 1998). In addition, physical disturbance (i.e.

wrack), interspecific competition, and root morphology can all influence primary and secondary succession in a salt marsh and be a major determinant of spatial patterns in marsh plant communities (Bertness and Ellison 1987, Bertness and Shumway 1993).

It is unclear whether the mechanisms that control vegetation patterns along the elevation gradient of a salt marsh, such as those described above, can be extended to describe the distribution of those same plants along the length of an estuary. A number of correlative studies have linked the longitudinal distributions of estuarine macrophytes to salinity. Higinbotham et al. (2004) identified four broad vegetation classes (salt marsh, brackish marsh, Juncus marsh, and fresh marsh) and observed that the upstream extent of these vegetation classes was generally related to average high tide salinity in Georgia estuaries. However, the vegetation in these marshes is heterogeneous, and Higinbotham et al. (2004) suggested that individual patches are probably responding to a larger set of complex factors that influence plant distribution and expansion. Clewell et al. (1999) found a relationship between the relative abundances of freshwater and salt tolerant vegetation and the maximum salinity that vegetation experienced in the Suwannee estuary in Florida, with a shift from *J. roemarianus* to *Cladium jamaicense* observed between 5 and 10 psu. Perry and Hershner (1999) identified a directional shift from tidal freshwater species (*Peltandra virginica*) to more salt-tolerant species (*S. cynosuroides*, *Carex hyalinolepis*) over a period of 13 years, which they speculated was a response to increasing salinities as a result of sea level rise.

Numerous experiments designed to explain the longitudinal distribution of marsh plants have been conducted in controlled, greenhouse environments. La Peyre et al. (2001) examined the influence of competition on three fresh and brackish marsh species

(*S. patens*, *Sagittaria lancifolia*, and *Panicum hernitomon*) that occur across a salinity gradient in the southeastern United States. This investigation took place in a greenhouse under four salinity treatments with the plants either alone or in three-species mixtures. Increasing salinity shifted community dominance toward the most salt tolerant species (*S. patens*), whereas competition altered community composition equally over all salinities. Interestingly, the relative importance of competition on an individual species' growth depended on its salinity tolerance, thus further underscoring the interaction of biotic and abiotic controls. In a South African estuary, Adams and Bate (1999) investigated field abiotic conditions (interstitial soil salinity, tidal inundation) in relationship to the distribution and production of the common reed *Phragmites australis*. They matched this non-manipulative field sampling with laboratory studies that assessed plant response to inundation with saline (35 psu) water aboveground and either 0 psu or 20 psu water below-ground. They found that plants that were supplied with fresh water to their roots grew and survived better than those supplied with 20 psu, suggesting that *P. australis* will only survive if their roots are located in brackish water. The above examples extend our understanding of plant response to changing conditions (i.e. salinity, competition, inundation). To our knowledge, only two manipulative field studies have studied how estuarine plants respond when transplanted from a low to a high salinity environment along an estuarine gradient. Kraemer et al. (1999) transplanted ramets of submerged aquatic vegetation (*Vallisneria americana*) to low, moderate and high salinity sites to investigate salinity tolerance and a transplant experiment in New England focused on transplanting marsh species between freshwater marshes and salt marshes (Mullen-Crain et al., in press).

Spartina alterniflora and *S. cynosuroides* are the codominant creekbank graminoids along the length of the Altamaha River estuary, GA (White 2004). *Spartina alterniflora* dominates in the high salinity marshes (salinity >15 psu); the two species overlap at intermediate salinities (~0.5-15 psu), and *S. cynosuroides* dominates in the oligohaline reaches (< 0.5 psu) until freshwater genera such as *Zizannia* become important. In this chapter, I investigate *Spartina* species distributions along the estuary to determine whether the controls described for *Spartina* distribution in a single salt marsh (physiological tolerance at the creekbank and competition at the upland border) can be applied along an estuarine gradient. Although physiological tolerance to salinity is often considered to be a key predictor of vegetation distribution (Adam 1990, Mitsch and Gosselink 1993), this has not yet been explicitly examined at the ecotone between *S. alterniflora* and *S. cynosuroides* along an estuarine salinity gradient. I was also interested in determining whether sulfate availability plays a role in *S. alterniflora* distribution as recent evidence suggests that *S. alterniflora* may have a greater than average sulfate nutrient requirement that is not met in brackish marsh environments (Ornes and Kaplan 1989, Stribling 1994, 1997). In this study, reciprocal transplants of *S. alterniflora* and *S. cynosuroides* were established along a 20 km length of the Altamaha River estuary. At the same time, neighbor presence (i.e. present or absent) was manipulated to assess competitive interactions.

METHODS

Study Site

This study was conducted along the creekbank of the Altamaha River estuary, GA, USA. The Altamaha River watershed is one of the largest on the East Coast with an area over 37,300 km^2. The estuary is approximately 54 km in length with an average width of 1.04 km and an average depth of 4.0 m (Dame et al. 2000). Salt marsh width in the estuary is approximately 12 km and tidal range is approximately 2-3 m (Dame et al. 2000). There is substantial freshwater discharge into the estuary (Alber and Sheldon 1999) and salt water is generally unable to penetrate far upstream. Salinity decreases from approximately 20 psu at the mouth of the estuary to 0 psu 20 km upstream (Alber and Sheldon 1999). However, over the course of this study (2001-2002) Georgia was experiencing a 4 year drought (1998-2002) and salinities 20 km upstream increased to approximately 15 psu (GCE/GA Rivers-LMER, unpublished data).

Higinbotham et al. (2004) used aerial photographs and GIS analyses to map the distribution of tidal marsh vegetation along the Altamaha River in coastal Georgia. They identified creekbank regions of salt marsh, which were dominated by *S. alterniflora* (up to 6 km from the mouth) and brackish marsh, which contained both *S. alterniflora* and *S. cynosuroides*, (between 6 and 16 km from the mouth). Ground vegetation surveys were conducted in 2000 and 2002 which clearly identified *S. alterniflora* and *S. cynosuroides* as the two codominant plant species found along the banks of the river (White 2004). Monotypic stands of *S. alterniflora* were located in high salinity environments up to ~ 7 km from the mouth of the river (typically > 15 psu); overlapping communities of *S. alterniflora* and *S. cynosuroides* occurred in mid-range salinity environments from ~ 7-15

km from the mouth (~0.5-15 psu), and monotypic stands of *S. cynosuroides* occurred in low salinity, brackish, environments beyond ~18 km from the mouth (typically < 0.5 psu). These bankside marshes are tidally inundated twice a day.

Reciprocal Transplant Experiment

Reciprocal transplants were performed to assess the growth potential of *S. alterniflora* and *S. cynosuroides* at the salinity extremes that exist along the Altamaha River (Figure 2.1). The salt marsh site was located approximately 2 km from the mouth and the brackish marsh site was 20 km further upstream. There were 40, 1.0-m^2 transplant plots spaced 2 m apart and 2- 3 m from the creekbank in each environment. Transplant sods were 30 x 30 cm wide and 30 cm in depth. Each sod initially contained approximately 4-6 similarly-sized *Spartina* ramets that were removed from monotypic stands of either *S. alterniflora* or *S. cynosuroides* growing on the creekbank and transplanted directly into either the opposite species' environment (N= 20) or back into the original environment (N=20) in February 2001. Of the 20 transplants in each environment, half were placed into plots with direct contact with surrounding vegetation (with competition), and in the remaining plots, surrounding vegetation within a radius of 35 cm from the transplant was removed monthly until aboveground regrowth did not occur (without competition). All treatments were systematically interspersed. Before initial survival was quantified in Spring 2001, all transplants were monitored for one month for transplant shock and replaced if they did not survive. Two of the *S. cynosuroides* transplants were found dead during this period. These were re-transplanted but did not survive and were not replaced. Unmanipulated control plots (N = 10) were also established in each zone.

Plant Performance

Measurements of transplant performance (growth and survival) as well as selected porewater constituents were assessed over the course of the experiment. Non-destructive measurements of transplant performance included the total number of shoots and seedheads per plot, plant height and tiller diameter of 10 randomly chosen plants in each plot (where available) and average leaf area of 5 plants (3 leaf lengths and widths were measured on 3 randomly selected leaves on each of the 5 plants). Initial measurements were taken in early April 2001 (one month after transplanting) and plants were then assessed in October 2001, March 2002 and at the end of the experiment in October 2002. Aboveground biomass was harvested in October 2002. Harvested plants were washed free of debris, rinsed with deionized water, dried at 60º C to constant weight, and both leaves and stems of randomly selected plants from each sample were ground in a Wiley Mill (40 µm mesh). Carbon, nitrogen, and sulfur concentrations were measured on ground plant tissue with a CE Elantech Flash Elemental Analyzer 1112. Samples were not acidified before CNS analysis because preliminary trials did not show significant differences in C, N or S between acidified and un-acidified samples. Acid extracts (Sah and Miller 1992) of selected transplants were analyzed for Al, Ca, Fe, K, Mg, Mn, Na, P, Si, and Sr on an inductively coupled plasma-atomic emission spectrophotometer (University of Georgia Chemical Analysis Lab). Plant samples that were collected for macro- and micronutrient analysis included both species in their natural habitat and their opposite transplant habitat. The number of samples selected for nutrient analysis depended on the number of live transplants available from each treatment within each zone. Samples from natural habitats included the control treatment (N=3) and plants

either with neighbors (N=3) or without neighbors (N=3) whereas samples from the opposite transplant habitat included transplants either with neighbors (N=1 for *S. alterniflora* and N=2 for *S. cynosuroides*) or without neighbors (N=3 for *S. alterniflora* and N=2 for *S. cynosuroides*).

Edaphic Parameters

Porewater samples were taken over the course of either one (ammonium, sulfate, and sulfide; May and October 2002) or two (salinity; April and October 2001, March and October 2002) growing seasons to better describe the transplant environment. Water samples were collected by installing 7 plastic PVC tubes (30.48 cm height x 5.72 cm diameter) into the substrate in each zone. The tubes were capped at both ends and had import ports at a depth of 15 cm which is where the majority of *Spartina* roots are located (Howes et al. 1981, DeLaune et al. 1983). Water was sampled from the tubes by first withdrawing all water from the well and then allowing them to refill if possible. If water did not recharge within the well then the initial water was stored for use. This was often the case, as creekbank soils generally drained quickly with little recharge into the wells in either transplant site. Interstital water was usually sampled during the ebb tide.

Pore water salinity was measured in the field, from samples drawn from the well, with a hand-held portable refractometer (Leica Model 10419, automatically temperature compensated). Dissolved sulfide was determined from water (10 ml) drawn directly from the well, filtered through 0.2 µm Gelman Acrodisc filter, and fixed in the field in separate collection bottles containing 0.05 M zinc acetate (5 ml) for colorimetric analysis (Cline 1969). The remainder of the sampled water obtained from the well (volume ranged from 10-120 ml) was collected in acid-washed containers, stored in a cooler, and transported

back to Sapelo Island where it was filtered (pre-combusted Whatman GF/F, 47 μm filter and a second filtering process through a 0.2 μm Gelman Acrodisc filter) and divided into aliquots for separate ammonium and sulfate analysis. Ammonium samples were stored frozen whereas sulfate and sulfide samples were refrigerated. Sulfate samples were acidified with 100 μL HNO_3 prior to refrigerating. Ammonium concentration was analyzed colorimetrically (Koroleff 1983) and measured with a spectrophotometer (Shimadzu UV-1601). Sulfate and sulfide were analyzed by M. Erickson and B. Porubsky in M. Joye's laboratory at the University of Georgia. Sulfate was analyzed by ion chromatography (EPA Method 300.1, Dionex LC20) and sulfide was measured spectrophotometrically (Cline 1969).

Data Analysis

Data were analyzed using analysis of variance (ANOVA), treating zone and treatment as fixed effects (Sokal and Rohlf 1995, SAS 2000). Initial plant measures were used in all cases as a standard covariate. A significance level (alpha) of 0.05 was used for all statistical analyses unless otherwise stated. All data were tested for normality (Shapiro-Wilk test statistic) and homogeneity of variance (Bartlett test statistic) prior to analysis. Where necessary, data were appropriately transformed to meet these assumptions. Tukey's post-hoc tests were used to examine the effect of zone and treatment on plant performance and edaphic parameters. Plant mortality is a nominal response variable (dead or alive) and logistic analyses were used to assess significance of zone and treatment on *Spartina* survival (SAS Institute 2000).

RESULTS

Transplant Environmental Conditions

Porewater salinities were significantly higher in the salt marsh than in the brackish marsh, averaging 11.46 ±1.05 and 3.33 ±0.20 practical salinity units (psu) respectively (p=<0.0001, Figure 2.2A). Salinities in the salt marsh were significantly lower during the spring than in the fall (Figure 2.3A) and showed much greater seasonal variation than those observed in the brackish marsh. Spring 2001 porewater salinities were lower than those observed in Spring 2002 in both marshes, which was likely a consequence of the extended drought conditions that existed in the southeast from 1999-2002. I used data from cruises along the Altamaha between 1999 and 2002 (GALMER and GALTER cruises) to calculate average high tide surface water salinities at each of the transplant sites over the course of the study. Average high tide surface water salinities at the salt marsh site were approximately 18 psu in 2000, 25 psu in 2001, and 24 psu in 2002. Brackish marsh average high tide surface water salinities were < 2 psu in 2000, < 3 psu in 2001, and < 1.5 psu in 2002.

Interstitial sulfate concentrations were also significantly greater in the salt marsh than in the brackish marsh, averaging 8.15 ± 0.85 and 0.84 ±0.34 mM respectively (p=<0.0001, Figure 2.2B). These concentrations are consistent with data from Stribling and Cornwell (2001) and Stribling (1994) who found interstitial sulfate concentrations in Maryland marshes to be between 1 and 2 mM at salinities of ~ 1 psu, 4-8 mM at mid-salinity conditions (~2-12 psu), and 8-14 mM at approximately 16 psu. Sulfate concentrations observed in the salt marsh in spring 2002 were significantly greater than those observed in fall 2002 (Table 2.1, Figure 2.3B) whereas no differences were

observed over the course of the observations in the brackish marsh. The early spring 2002 maximum in sulfate concentration in the salt marsh is consistent with the low temperatures and more oxidized sediments (from early growth plant root oxidation of sediments) often observed in winter and early spring in marsh sediments. This type of environment can result in increased production of sulfate from any sulfide minerals previously chemically bound in the sediment (Howes 1985, Matson and Brinson 1985). As the growing season progresses, increased temperatures during the summer and fall are generally associated with greater sediment oxygen consumption which would decrease interstitial sulfate concentrations.

In contrast to sulfate, sulfide concentrations did not exhibit significant differences between sites, although the averages were higher in the salt marsh (293.22 ± 139.84 μM) as compared to the brackish marsh (130.89 ± 51.11 μM; Figure 2.2C and 2.3C). The sulfide concentrations observed in the salt marsh were similar to values found by Baldwin and Mendelssohn (1998) in Louisiana in saline conditions (223.33 ± 1.33 μM). The brackish marsh values were similar to reports in low salinity environments (Stribling and Cornwell 2001) and higher than reports in freshwater marshes (Baldwin and Mendelssohn 1998). Low sulfide concentrations at rooting depths, such as observed in the brackish marsh, suggest that available sulfate is not being reduced to sulfide to an extent that would hinder *S. alterniflora* growth (Howarth et al. 1983). However, Bradley and Dunn (1989) found that *S. cynosuroides* growth was hindered at sulfide concentrations greater than 0.5 mM, so it is possible that the reduced survival of this species in the salt marsh is linked to both salinity and sulfide conditions. We also observed an increase in interstitial sulfide later in the growing season which occurred at

the same time as a decrease in sulfate. This was likely a result of reduced oxygen availability in the fall, as decreases in root zone oxidation occurs due to a reduction in above and belowground growth (Hines et al. 1989) and/or the stimulation of sulfate reduction by the increasing organic matter created as plants senesce (Howarth and Teal 1979).

There were also no differences in ammonium concentrations between the two creekbank zones either in terms of overall average concentrations (96.77 ±17.96 μM in the salt marsh and 136.37 ±51.76 μM in the brackish marsh) or in terms of seasonal cycles (Figure 2.2D and 2.3D). Mendelssohn (1979) found that ammonium concentrations ranged from 42.2 to 242.36 μM depending on whether the samples were taken in tall or short *S. alterniflora* stands, respectively. Stribling and Cornwell (2001) found little variability in ammonium concentrations between low, medium and high salinity environments (56.69, 31.41, and 46.57 μM, respectively). The lack of differences between zones in both ammonium and sulfide concentrations may be a result of the large daily tidal flushing both sites experience. Sufficient flushing can not only remove ammonium from the *Spartina* root zones via advection, but this movement of water through creekbank sediment can also influence sediment biogeochemical processes by alleviating anoxic conditions (Howes et al. 1981, Mendelssohn et al. 1981, King et al. 1982, Howarth et al. 1983, Howes et al. 1986).

Survival

When transplanted out of their own zone, survival of both species was significantly reduced over the course of the two growing seasons (Figure 2.4). *Spartina alterniflora* survival at the end of the experiment averaged 20% in the brackish marsh and

80% in the salt marsh whereas *S. cynosuroides* survival averaged 20% in the salt marsh and 60% in the brackish marsh (Table 2.2A and 2.2B). The presence of neighbors had no effect on the survival of either species in the salt marsh. In the brackish marsh, survival of both *S. alterniflora* and *S. cynosuroides* was lower in treatments where neighbors remained as compared to those where neighbors were removed, (although these differences were not significant; Table 2.2A). All plants in control plots within each zone survived over the course of the experiment.

Plant Metrics

Average height, number of leaves, leaf area, tiller diameter, and number of plants of initial *S. alterniflora* transplants were similar to those of initial *S. cynosuroides* transplants. Final plant growth parameters were first compared with regard to transplant environment (brackish versus salt marsh), regardless of the presence or absence of neighbors, to evaluate the importance of abiotic conditions on plant performance (Figure 2.5). Each species performed better in its own environment, which is consistent with the survival data (Table 2.3). *Spartina alterniflora* final plant height, tiller diameter, number of leaves, and aboveground biomass were all significantly greater in the salt marsh than they were in the brackish marsh. *Spartina cynosuroides* had significantly more plants in the brackish marsh as compared to the salt marsh and, although not significant, the trend of greater values in the brackish marsh held for all other plant metrics. In addition, each species outperformed the other in its own environment for most plant metrics (Table 2.4). *Spartina alterniflora* growth metrics were significantly greater than those of *S. cynosuroides* in the salt marsh for all measured parameters except tiller diameter, number of leaves, and leaf area. *S. cynosuroides* growth metrics were greater than *S. alterniflora*

in the brackish marsh for all measured parameters except leaf area and number of plants. Although not significant for the remaining plant metrics, both *S. alterniflora* and *S. cynosuroides* tended to have larger measurements in their native zone when compared to the reciprocal transplants into that zone.

Spartina alterniflora tissue nutrient concentrations were comparable to previously reported values (Gallagher 1975, Linthurst and Seneca 1981, Ornes and Kaplan 1989, Stribling and Cornwell 2001). Although there is little tissue nutrient information available for *S. cynosuroides*, one study reported an average N content of *S. cynosuroides* of 0.30 % (Beale and Long 1997) while another reported a leaf value of 0.66 % N (Hopkinson and Schubauer 1984). These reported values are lower than our observed *S. cynosuroides* % N concentrations which range from 0.85 ± 0.02 (unmanipulated *S. cynosuroides* plants in the brackish marsh) to 1.1 ± 0.1 (*S. cynosuroides* transplants without neighbors in the brackish marsh). *Spartina* tissue nutrient concentrations showed differences between marsh habitats (Figure 2.6, Table 2.3). Both species had significantly higher N:S (mol/mol) ratios in the brackish as compared to the salt marsh environment. For both species, the change in N:S ratios were coupled to significant decreases in % S in the brackish marsh. There were no differences in any other parameters (% C, C:N) between the sites for either plant. There were also differences between the two species: *S. cynosuroides* had significantly higher % C and lower % N than *S. alterniflora* in both the salt and brackish marsh environments which led to higher C:N ratios (Table 2.4). *Spartina cynosuroides* is a taller, wider plant than *S. alterniflora*, with more structural material, so the fact that its % C and C:N values were higher was not

unexpected. The only other difference between the species was that *S. alterniflora* had a higher % S than *S. cynosuroides* in both the brackish and salt marsh.

We also assessed *S. alterniflora* and *S. cynosuroides* macro- and micronutrient concentrations of transplants from each zone (Table 2.5). There were no tissue nutrient differences in *S. alterniflora* transplants from the salt marsh as compared to the brackish marsh environment. There were, however, differences in *S. cynosuroides* plant tissue content between the salt and brackish marsh environment for a number of elements. *Spartina cynosuroides* transplants had higher concentrations of Al, Fe, K, Mg, Mn, Na, and Si in the salt marsh as compared to the brackish marsh environment; only Mn concentrations were significantly lower in the salt marsh.

Transplant performance was analyzed by neighbor treatment (presence, absence, unmanipulated control) to explore how biotic conditions (inter- and intraspecies interactions), in conjunction with location (brackish versus salt marsh environment), affected *Spartina* growth (Figures 2.7 and 2.8). *Spartina alterniflora* exhibited a strong positive response to neighbor removal in the salt marsh: final height, leaf area, number of leaves, biomass, and number of plants were all significantly greater than when neighbors were present (and both total height and number of plants were greater than control plants; Figure 2.7, Table 2.6A). In contrast, *S. alterniflora* showed little response to the presence or absence of *S. cynosuroides* neighbors in the brackish marsh (Figure 2.7, Table 2.6B): only tiller diameters were significantly increased in treatments where neighbors were removed. Given that *S. alterniflora* survival and performance was diminished in the brackish marsh as compared to the salt marsh (Figures 2.4 and 2.5), it may be that biotic effects are secondary. *Spartina cynosuroides* showed no statistically significant

differences in plant performance with or without neighbors in either the salt or brackish marsh environment (Figure 2.8, Table 2.6C). It should be noted, however, that *S. cynosuroides* biomass was significantly greater in the control, unmanipulated, plot as compared to either treatment (Figure 2.8, Table 2.6D). It is possible that the act of transplanting (regardless of neighbor presence) could have had a negative impact on aboveground plant production.

Finally, we evaluated how the presence of neighbors might affect the observed differences in the performance of the two *Spartina* species in each habitat (Table 2.7). Differences were not apparent when neighbors were present in either environment. However, when neighbors were removed, each species out-performed the other in its own environment. In the salt marsh, most *S. alterniflora* metrics were significantly greater than those measured in *S. cynosuroides* when neighbors were removed (all except tiller diameter and number of leaves). In the brackish marsh, *S. cynosuroides* tiller diameter and number of leaves were significantly greater than *S. alterniflora* when neighbors were removed. Thus, the improved plant performance in its own zone was only evident when plants were alone (not competing). These differences disappeared when neighbors were present.

DISCUSSION

The mechanisms controlling the distribution of marsh vegetation along the length of an estuary are not well understood. If the general salt marsh paradigm were applicable over the longitudinal gradient of an estuary, then we would predict that *S. cynosuroides* is physiologically excluded from the salt marsh environment and *S. alterniflora* competitively excluded from the brackish marsh. However, if *S. alterniflora* were to

have a sulfate requirement that is not being met in the brackish marsh, our results should show reduced growth of this species at low salinities, resulting from reduced sulfate availability. In terms of biotic interactions, we might anticipate finding facilitative interactions in the harsher, salt marsh environment and competitive interactions in the more benign, brackish marsh. If this were the case, *S. cynosuroides* might do poorly in the salt marsh without neighbors whereas *S. alterniflora* would survive best in the brackish marsh without neighbors. The results of this study only partially support these predictions.

Abiotic Effects on Spartina Success

The fact that each *Spartina* species survived and performed better in its native, original environment suggests that the conditions within a particular marsh environment (salt or brackish) affords some type of specific benefit for the native species. This is further supported by the observation that those reciprocal transplants that did survive in the opposite environment always did poorly as compared to the natural transplants in that zone. These results fit the predictions for *S. cynosuroides* but not for *S. alterniflora*. It should be noted that mortality in the transplants was progressive over the course of the experiment (Figure 2.4) and thus it is unlikely that these plants died due to transplant shock.

It is possible that the poor *S. cynosuroides* performance observed in the salt marsh is linked to both the higher salinity and sulfide concentrations at this site. Since *S. cynosuroides* is generally restricted to lower salinity areas it is reasonable to assume that this species is physiologically incapable of surviving in the higher salinities found in the salt marsh and would perform best in the lower salinity areas where it is the dominant

species. This fits the prediction that harsh abiotic conditions set the downstream limit of *S. cynosuroides*. When we examined the elemental composition of *S. cynosuroides* in the two environments, we found that the concentrations of many macronutrients (Al, Fe, K, Mg, Na, and Si) were greater in the salt marsh as compared to the brackish marsh. Little information is available regarding the elemental composition of *S. cynosuroides*, but our observations were generally within range of published values for *S. alterniflora* (Table 2.8). One element (Mn) was present in much higher concentrations than reported values (Broome et al. 1975, Gallagher 1975, Ornes et al. 1998). It is unclear if *S. cynosuroides* has a higher Mn content than *S. alterniflora* naturally, or if these large concentrations are hindering *S. cynosuroides* plant growth in the salt marsh.

The survival of *S. alterniflora* was unexpectedly poor in the brackish marsh. It is unlikely that this was due to the low salinity conditions, as numerous studies have demonstrated that *S. alterniflora* grows best at low salinities (Adams 1963, Haines and Dunn 1976, Smart and Barko 1980, Cavalieri 1983, Drake and Gallagher 1984). The greater survival and growth of *S. alterniflora* in the higher salinity environment observed here suggests that there is some other constraint in the brackish marsh environment that prevents *S. alterniflora* from succeeding in the lower salinity area.

Interstitial sulfate concentration may influence the upper limit of *S. alterniflora* distributions. In this study, interstitial sulfide and sulfate concentrations were significantly lower in the brackish marsh as compared to the salt marsh. This is not surprising given the high sulfate concentration found in seawater. It has been suggested that *S. alterniflora* might have a physiological requirement for sulfate that is not fulfilled in brackish environments (Ornes and Kaplan 1989, Stribling 1994). Where sulfate is in

short supply, *S. alterniflora* uptake and dissimilatory sulfate reduction may compete (Ponnamperuma 1972, Stribling 1994, 1997), possibly leading to decreased growth. Stribling (1997) suggested that *S. alterniflora* growth was hindered in environments where sulfate concentrations were below ~ 0.5-1.0 mM. The porewater sulfate samples we observed in the brackish marsh (0.84 ± 0.34 mM) were within this low range, and may account, in part, for the poor *S. alterniflora* performance we observed.

In order to further consider the potential influence of low porewater sulfate concentrations on *S. alterniflora*, we examined tissue nutrient concentrations. We reasoned that, if *S. alterniflora* growth and survival was hindered through a lack of required sulfate in the brackish marsh, then this would be reflected in the tissues (Bradley and Morris 1991). The % S content of *S. alterniflora* transplants ranged between 0.34 and 0.51 % in both environments, but the values were significantly lower in brackish versus salt marshes. These values are low when compared to reported % S as high as 1.2 % in salt marshes (Carlson 1980, Carlson and Forrest 1982, Ornes and Kaplan 1989). Our observations were consistent with those of Stribling and Cornwell (2001) who reported *S. alterniflora* % S values that ranged from 0.39-0.45 in brackish conditions and 0.44-0.50 % in more saline marshes.

While lower tissue sulfur concentrations did co-occur with reduced *S. alterniflora* growth in the brackish marsh, there seems to be adequate nitrogen available for successful growth in both environments. Smart and Barko (1980) suggested that the adequate percent nitrogen nutrition for *S. alterniflora* ranges between 0.66-0.80 %; our *S. alterniflora* nitrogen tissue values surpass this range in both marsh environments. However, N:S molar ratios of *S. alterniflora* in this study increased from 6.01 (±0.35) in

the salt marsh to 9.99 (±3.07) in the brackish marsh. A similar increase in *S. cynosuroides* N:S ratios in the brackish marsh as compared to the salt marsh was also observed. This increase in the brackish marsh was a result of *S. alterniflora*'s increase in nitrogen content with a concurrent decrease in sulfur content. Previous work has shown that plants with N:S ratios as high as 30:1 are not deficient in sulfur (Epstein 1972), although typical ratios are about 20:1 (Tabatabai 1984). Stribling (1994) and Stribling and Cornwell (2001) reported N:S ratios of less than 3:1 in a low salinity Maryland environment and ratios approximately 5:1 in higher salinity environments. The low % S in the brackish marsh and higher N:S ratios are both consistent with a sulfate requirement limiting *S. alterniflora*'s upper estuarine distribution. Other micro- and macronutrient concentrations did not vary between environments and were within the range of reported values (Table 2.8), so this did not provide any additional insight into controls on this species growth. It is important that additional sulfate experiments be conducted to further explore the potential of sulfate to control *S. alterniflora* performance.

Plant-Plant Interactions Effects on Spartina Success

In the salt marsh zone, neighbor presence had a negative influence on *S. alterniflora* growth (i.e. intraspecific competition) as evidenced by decreases in plant growth parameters in treatments where neighbors were present (height, number of leaves, leaf area, and biomass). For two of the dependent variables measured (height and number of plants), treatments with neighbors removed were significantly greater than unmanipulated controls, suggesting stimulation of growth with neighbors absent in addition to reduction in the treatments with neighbors present. Rather than a facilitation effect, which might be expected in a harsh environment (Bertness and Shumway 1993,

Bertness and Yeh 1994, Bertness and Hacker 1994, Bruno 2000, Bruno and Kennedy 2000), this pattern of reduced growth of *S. alterniflora* transplants when neighbors are present suggests intraspecific competition for resources. It is likely that the resources that are limiting for *S. alterniflora* are available space and light rather than nitrogen. Tissue % N concentrations were greater than the range proposed by Smart and Barko (1980) for adequate nitrogen content.

In contrast to the differences in plant performance, neighbor presence did not influence survival of *S. alterniflora* in the salt marsh. This seemingly contradictory response between different components of fitness (survival versus growth) has been explored by Goldberg and Novoplansky (1997). Neighbors may offer protection to one another in severe environments, thus increasing the probability of their survival, however, these same neighbors will also be competing for available resources and this results in decreased growth. Levine (2000) found that *Carex* neighbors in a California riparian community facilitated survival by protecting one another from winter disturbances, yet these plants also competed with one another such that overall biomass was reduced. In some coastal dune environments in Georgia and Florida, Franks (2003) found that the presence of *Uniola* or *Iva* neighbors reduced target plant biomass while increasing the likelihood of that plant's survival. Neighbor presence did not significantly reduce *S. alterniflora* survival compared to when neighbors were absent, yet we do find reduced biomass for this species when neighbors are present. This suggests that, although growth is constrained by neighbor presence, the probability of survival may be increased due to these neighbors.

There is no evidence that neighbor presence or absence influenced *S. cynosuroides* growth in the salt marsh, which suggests that the physical stress experienced by *S. cynosuroides* plants in this environment overshadowed any facilitative interactions that might have contributed to increased survival and growth. It should be noted, however, that since *S. cynosuroides* transplant survival was so poor in the salt marsh environment, the influence of the presence or absence of *S. alternilfora* neighbors on growth could not be statistically differentiated.

Both species showed increased mortality when neighbors were present in the brackish marsh (10% survival for *S. alterniflora* and 40% for *S. cynosuroides*), suggesting that competition might indeed influence plant success in the lower salinity environment. However, this was not observed in growth metrics for either species. We expected *S. alterniflora* to do well when neighbors were removed in the brackish marsh, since competitive displacement to harsher environments by less tolerant, more competitively able species defines *S. alterniflora*'s presence in the salt marsh. However, we were unable to statistically evaluate whether interspecific competition is a mechanism that hinders *S. alterniflora*'s growth in the brackish marsh as our high transplant mortality in this area reduced our statistical power. The only growth metric that we can point to that suggests competitive interactions is tiller diameter, where neighbor presence reduced tiller diameter in the brackish marsh (Figure 2.7). We might expect to find intraspecific competition between *S. cynosuroides* plants in the brackish marsh as these plants would access similar resources and the environment is not harsh enough to foster more facilitative interactions. There were no neighbor effects, however, for *S. cynosuroides* transplants in the brackish marsh. Thus we are unable to make a case for increased

competitive interactions between species in the brackish marsh as compared to the salt marsh environment.

Evaluating Mechanisms of Distribution Control along the Length of an Estuary

The results of this study suggest that the distribution of *Spartina* species is maintained primarily by the abiotic environment, as indicated by the observation that neither species survived well when transplanted to the opposite environment, regardless of neighbor presence (Figures 2.3 and 2.4). Reduced growth and survival of *S. cynosuroides* in the salt marsh environment is likely the result of increased salinities, although increased porewater sulfides may have also been a factor. It is also possible that there was some additional macro- or micronutrient limitation or even toxicity (i.e. Mn). Additional research is needed on this topic. Reduced growth and survival of *S. alterniflora* in the brackish marsh may be due to a sulfate requirement, as evidenced by decreased % S and increased N:S in the brackish environment. Our results are similar to work completed in a Maryland marsh (Stribling 1994, Stribling and Cornwell 2001). Their assertion that limiting sulfate conditions may control the upper distribution of *S. alterniflora* is supported by our work. However, greenhouse sulfate addition experiments where sulfate concentrations range from conditions expected at fresh, brackish, and salt marsh levels could aid our understanding of sulfate requirements that could be missed in the field where many abiotic and biotic interactions contribute to vegetation patterns (Chapter 3).

Spartina cynosuroides showed little response to the presence or absence of neighbors in this experiment. In contrast, neighbor presence had a strong negative influence on *S. alterniflora* success in the salt marsh. In fact, the improved growth and

survival of *S. alterniflora* in the salt marsh environment was only observed in the absence of neighbors. This suggests a role for intraspecific competition regulating the population of *S. alterniflora* in its native environment. Interestingly, this interaction was not consistent with the general theory of reduced competition with greater environmental stress (Davy and Smith 1985, Bertness and Ellison 1987, Bertness 1991a, b). It is possible that there is a genetic component to *S. alterniflora*'s ability to adapt to different environmental conditions. Hester et al. (1996, 2001) showed that under short term (< 3 months) sublethal salinity stress, *S. alterniflora* exhibits intraspecies genetic variation in salt tolerance. The genetic differences between and among *Spartina* species along estuarine gradients has not been investigated. A more thorough understanding of this species genetic makeup would be a useful addition to better defining *Spartina* distributional controls along an estuary.

A growing body of investigations are being conducted to determine how well single marsh manipulative results can be generalized across geographic ranges (Pennings and Bertness 1999, Pennings and Bertness 2001, Pennings and Moore 2001, Bertness and Ewanchuk 2002, Pennings et al. 2003). Extrapolating, or generalizing, experimental results from small scale or single site research efforts to broader spatial scales has challenged ecologists for decades. Time and resources are limited, such that investigating every species and community separately is unreasonable; hence, the appeal of being able to extrapolate results from well-studied to poorly-studied systems. Generalizing results from one site to another is challenging in a number of ways; the nature of biotic interactions may change with abiotic conditions (Dunson and Travis 1991) and over extrapolation of results may occur, leading to incorrect application of

scientific knowledge (Underwood and Denley 1984). The results reported here suggest that it is inappropriate to generalize mechanisms that control distributions in a salt marsh out to the broader scale of an estuarine salinity gradient. If the mechanisms controlling the upper estuarine limits of *S. alterniflora*'s distribution can be better defined, this information, in combination with our understanding of *S. cynosuroides* distributions, will enable us to continue to scale up from single site studies (this estuary) to cross-site comparison studies (another estuary in the southeast or northeast), thus increasing our understanding of variations between and among estuaries.

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Table 2.1. Edaphic parameters over the course of the experiment in each zone and averaged by zone. F and p-values are from ANOVAs and indicate significant differences between the salt and brackish marsh zones.

Nutrient	**Salt marsh Mean ± S.E. (N)**	**Brackish marsh Mean ± S.E. (N)**	**F**	**p-value**
NH4 (µM)				
Sp 02	43.4 ± 19.3 (7)	5.7 ± 2.3 (3)	1.52	0.2521
Sum 02	92.5 ± 28.0 (9)	216 ± 113 (6)	1.61	0.2261
Fl 02	142 ± 33 (9)	121 ± 52 (6)	0.13	0.7276
Average	96.7 ±17.9 (25)	136 ±51 (15)	0.74	0.3955
SO4 (mM)				
Sp 02	11.6 ± 1.5 (4)	1.1 ± 0.7 (2)	19.14	**0.0119**
Sum 02	7.8 ± 0.5 (5)	0.9 ± 0.6 (5)	66.73	**<0.0001**
Fl 02	5.6 ± 0.8 (5)	0.4 ± 0.0 (3)	20.21	**0.0041**
Average	8.1 ±0.8 (5)	0.8 ±0.3 (10)	48.48	**<0.0001**
H2S (µM)				
Sp 02	120 ± 75 (4)	8.5 ± 5.0 (3)	1.56	0.2669
Sum 02	300 ± 282 (7)	62.4 ± 35.0 (6)	0.60	0.4567
Fl 02	384 ± 238 (7)	286 ± 110 (5)	0.11	0.7502
Average	293 ± 139 (18)	130 ± 51 (14)	0.96	0.3341
Salinity (psu)		.		
Sp 01	4.2 ± 0.33 (23)	2.6 ± 0.3 (20)	12.59	**0.0010**
Fl 01	20.0 ± 0.3 (10)	3.4 ± 0.2 (9)	1163.07	**<0.0001**
Sp 02	13.1 ± 0.8 (10)	4.3 ± 0.3 (9)	97.75	**<0.0001**
Fl 02	20.5 ± 0.5 (7)	4.2 ± 0.5 (5)	381.07	**<0.0001**
Average	11.4 ± 1.0 (50)	3.3 ± 0.2 (43)	50.47	**<0.0001**

Table 2.2A. Logistic regression table for survival of reciprocal transplants (N=50).

Species **Main Effect**	**df**	**Wald χ^2**	**P**
S. alterniflora			
Zone	1	10.57	**0.0011**
Treatment	1	1.25	0.2629
S. cynosuroides			
Zone	1	4.98	**0.0256**
Treatment	1	1.08	0.2974

Table 2.2B. Percent survival of transplants in each zone and by neighbor treatment.

Transplant	**Salt marsh**	**Brackish marsh**
S. alterniflora	80%	20%
With neighbors	80%	10%
Without neighbors	80%	30%
S. cynosuroides	20%	60%
With neighbors	20%	40%
Without neighbors	20%	80%

Table 2.3. Final transplant growth parameters and tissue nutrient concentrations in A) *S. alterniflora* and B) *S. cynosuroides* in the salt and brackish marsh environments. F and p-values are from ANOVAs and indicate significant differences between the salt and brackish marsh zones.

A. *S. alterniflora*

	Salt Marsh Mean ± S.E. (N)	**Brackish Marsh Mean ± S.E. (N)**	**F-value**	**P-value**
Growth Parameters				
Height (m/plot)	15.5 ± 3.4 (15)	1.8 ± 0.6 (4)	6.06	**0.0248**
Tiller (mm/plot)	34.7 ± 2.9 (15)	11.5 ± 3.2 (4)	11.61	**0.0036**
No. Leaves/per plot	37.9 ± 5.1 (14)	14.3 ± 6.9 (4)	5.12	**0.0401**
Leaf area (cm^2/plot)	1744 ± 269 (14)	388 ± 134 (2)	2.71	0.1220
Biomass (g/plot)	108 ± 26 (15)	7.1 ± 4.5 (2)	9.28	**0.0073**
Seedheads/plot	5.8 ± 1.33 (8)	0	NA	NA
No. Plants/plot	16 ± 3.1 (15)	3.3 ± 1.3 (3)	3.82	0.0684
Tissue Nutrients				
% Nitrogen	1.4 ± 0.08 (7)	1.8 ± 0.2 (4)	3.06	0.1142
% Carbon	41.3 ± 0.8 (7)	41.0 ± 0.5 (4)	0.04	0.8384
% Sulfur	0.58 ± 0.03 (7)	0.42 ± 0.03 (4)	12.38	**0.0065**
C:N	33.6 ± 4.0 (7)	28.6 ± 4.6 (4)	0.60	0.4589
N:S	5.5 ± 0.3 (7)	9.9 ± 1.5 (4)	14.23	**0.0044**

2.3B. *S. cynosuroides*

	Salt Marsh Mean (± S.E.); N	Brackish Marsh Mean (± S.E.); N	F-value	P-value
Growth Parameters				
Height (m/plot)	1.9 ± 0.7 (4)	9.5 ± 2.8 (12)	3.78	0.0721
Tiller (mm/plot)	12.6 ± 2.8 (3)	24.2 ± 2.9 (12)	2.00	0.1813
No. Leaves/per plot	9.6 ± 2.9 (3)	23.1 ± 3.2 (12)	4.11	0.0635
Leaf area (cm^2/plot)	289 ± 98 (3)	771 ± 177 (12)	2.42	0.143
Biomass (g/plot)	8.9 ± 3.3 (4)	28.9 ± 7.9 (11)	2.50	0.1376
Seedheads/plot	0	1 (1)	NA	NA
No. Plants/plot	1.8 ± 0.4 (4)	8.8 ± 2.1 (12)	5.39	**0.0359**
Tissue Nutrients				
% Nitrogen	0.83 ± 0.07 (11)	1 ± 0.1 (6)	0.98	0.3547
% Carbon	44.3 ± 0.4 (11)	44.3 ± 0.5 (6)	0.00	0.9834
% Sulfur	0.39 ± 0.05 (11)	0.21 ± 0.03 (6)	11.34	**0.0120**
C:N	63.0 ± 5.7 (11)	55.5 ± 7.3 (6)	0.43	0.5331
N:S	5.0 ± 0.3 (11)	10.9 ± 0.5 (6)	62.68	**<0.0001**

Table 2.4. Results of t-tests comparing growth parameters and tissue nutrient concentrations of *S. alterniflora* and *S. cynosuroides* in A) Salt marsh and B) Brackish marsh environments. Differences between species within an environment are indicated by boldface.

	Salt marsh	**Brackish Marsh**
Metric	**P**	**p**
Height (m/plot)	**0.0458**	**0.0340**
Tiller (mm/plot)	0.2017	**0.0121**
No. Leaves/ plot	0.0878	**0.0223**
Leaf area (cm^2/plot)	0.0533	0.2526
Biomass (g/plot)	**0.0214**	**0.0492**
No. Plants/plot	**0.0335**	0.0736
% Nitrogen	**0.0042**	**0.0175**
% Carbon	**0.0389**	**0.0030**
% Sulfur	**0.0105**	**0.0010**
C:N	**0.0036**	**0.0259**
N:S	0.3016	0.5270

Table 2.5. *S. alterniflora* (SA) and *S. cynosuroides* (SC) macronutrient values (mean ± S.E.) from both reciprocal environments (N=3). Letters indicate differences between salt and brackish marsh environments within a species. Highest significance level of t-test comparisons between *S. alterniflora* and *S. cynosuroides* tissue concentrations in each environment is indicated by asterisks; *-p<0.05; **-p<0.01; ***-p<0.001. (ns= not significant and nd=not detectable)

Nutrient (ppm)	**SA Zone N=3**	**SC Zone N=3**
Al	ns	ns
SA	1542 ± 433	3047 ± 1708
SC	2051 ± 78a	110 ± 25b
Ca	**	*
SA	3385 ± 343	3190 ± 581
SC	930 ± 156	1140 ± 136
Fe	ns	ns
SA	939 ± 240	1861 ± 884
SC	1129 ± 43a	268 ± 28b
K	*	***
SA	9470 ± 1682	10031 ± 171
SC	3519 ± 226.12a	1374 ± 385b
Mg	**	**
SA	4774 ± 421	4391 ± 256
SC	2449 ± 156a	1057 ± 194b
Mn	**	ns
SA	257 ± 16	499 ± 117
SC	87 ± 11b	349 ± 40a
Na	**	**
SA	9551 ± 928	6581 ± 941
SC	4290 ± 530a	2043 ± 173b
P	*	*
SA	1667 ± 254	2335 ± 422
SC	720 ± 85	666 ± 98
Si	ns	*
SA	2477 ± 296	2865 ± 863
SC	1687 ± 17a	428 ± 40b
Sr	**	
SA	51.6 ± 4.0	36.0 ± 4.1
SC	15.6 ± 1.5	nd

Table 2.6. A. *S. alterniflora* and B. *S. cynosuroides* final transplant growth and tissue nutrient values by treatment within the salt and brackish marsh environment. F and p-values are from ANOVAs and indicate significant differences between neighbor treatments.

A. *S. alterniflora* in the Salt Marsh; treatments are without neighbors (WO), with neighbors (WN) and the unmanipulated control (UC). Different letters indicate significant differences between treatments in the salt marsh ($P<0.05$, Tukey's post hoc test).

Metric	WO Mean ± S.E. (N)	WN Mean ± S.E. (N)	UC Mean ± S.E.; (N)	F-value	P-value
Height (m/plot)	25.54^{a} ± 0.37 (8)	4.0^{b} ± 1.1 (7)	9.9^{b} ± 0.65 (4)	17.85	**<0.0001**
Tiller (mm/plot)	42.38^{a} ± 1.55 (8)	25.8^{a} ± 4.1 (7)	42.5^{a} ± 1.3 (4)	10.93	0.3757
No. Leaves/ plot	53.14^{a} ± 3.02 (7)	22.7^{b} ± 5.0 (7)	46.5^{ab} ± 1.5 (4)	17.4	**0.0212**
Leaf area (cm^2/plot)	2354^{a} ± 234 (7)	1133^{b} ± 366 (7)	2448^{ab} ± 191 (4)	6.04	**0.0062**
Biomass (g/plot)	180^{a} ± 28 (8)	26.9^{b} ± 8.56 (7)	100^{a} ± 20 (4)	13.12	**0.0012**
No. Plants/plot	26.0^{a} ± 2.1 (8)	4.5^{b} ± 1.0 (7)	6.0^{b} ± 0.0 (4)	51.44	**0.0002**
% Nitrogen	1.3^{a} ± 0.1 (2)	1.4^{a} ± 0.1 (5)	1.1^{a} ± 0.1(3)	1.61	0.2650
% Carbon	42.4^{a} ± 0.3 (2)	40.8^{a} ± 0.9 (5)	42.1^{a} ± 0.7 (3)	0.86	0.4629
% Sulfur	0.52^{ab} ± 0.04 (2)	0.60^{a} ± 0.04 (5)	0.37^{b} ± 0.03 (3)	10.01	**0.0089**
C:N	33.4^{a} ± 5.8 (2)	29.5^{a} ± 5.1 (5)	38.1^{a} ± 5.4 (3)	1.52	0.2834
N:S	5.7^{a} ± 1.2 (2)	5.4^{a} ± 0.1 (5)	7.0^{a} ± 0.6 (3)	2.82	0.1263

2.6B. *S. alterniflora* in the Brackish Marsh; treatments are without neighbors (WO) and with neighbors (WN). Different letters indicate significant differences between treatments in the brackish marsh ($P<0.05$, Tukey's post hoc test).

Metric	WO Mean ± S.E. (N)	WN Mean ± S.E. (N)	F-value	P-value
Height (m/plot)	$2.3^{a} \pm 0.7$ (3)	0.6^{a} (1)	1.2	0.0542
Tiller (mm/plot)	$14.0^{a} \pm 3$ (3)	4^{b} (1)	2.78	**0.0032**
No. Leaves/ plot	$16.0^{a} \pm 9.5$ (3)	9^{a} (1)	0.14	0.748
Leaf area (cm^2/plot)	$479^{a} \pm 140$ (3)	115^{a} (1)	1.69	0.323
Biomass (g/plot)	$9.4^{a} \pm 5.5$ (3)	0.23^{a} (1)	0.7	0.4921
No. Plants/plot	4^{a} (2)	2^{a} (1)	0.33	0.575
% Nitrogen	$1.5^{a} \pm 0.21$ (3)	2.56^{a} (1)	5.8	0.1378
% Carbon	$40.9^{a} \pm 0.7$ (3)	41.43^{a} (1)	0.11	0.770
% Sulfur	$0.41^{a} \pm 0.04$ (3)	0.42^{a} (1)	0	0.962
C:N	$31.9^{a} \pm 4.6$ (3)	18.84^{a} (1)	1.99	0.294
N:S	$8.6^{a} \pm 1.0$ (3)	13.99^{a} (1)	6.02	0.133

2.6C. *S. cynosuriodes* in the Salt Marsh; treatments are without neighbors (WO) and with neighbors (WN). No differences exist between treatments in the salt marsh.

Metric	WO Mean (± S.E.); N	WN Mean (± S.E.); N	F-value	P-value
Height (m/plot)	1.7 ± 1.6 (2)	2.2 ± 0.7 (2)	0.1	0.8858
Tiller (mm/plot)	16 (1)	11 ± 4 (2)	0.52	0.9057
No. Leaves/ plot	14 (1)	7.5 ± 3.5 (2)	1.15	0.4409
Leaf Area (cm^2/plot)	353 (1)	257 ± 161 (2)	0.12	0.2291
Biomass (g/plot)	7.8 ± 7.6 (2)	10.0 ± 2.2 (2)	0.08	0.5216
No. Plants/plot	2 ± 1 (2)	1.5 ± 0.5 (2)	0.2	0.6164
% Nitrogen	0.96 (1)	0.77 ± 0.07 (2)	2.45	0.3617
% Carbon	44.9 (1)	44.0 ± 0.6 (2)	0.76	0.544
% Sulfur	0.47 (1)	0.35 ± 0.06 (2)	1.56	0.430
C:N	54.6 (1)	67.2 ± 6.9 (2)	1.09	0.4864
N:S	4.64 (1)	5.1 ± 0.4 (2)	0.54	0.5972

2.6D. *S. cynosuroides* in the Brackish Marsh; treatments are without neighbors (WO), with neighbors (WN) and the unmanipulated control (UC). Different letters indicate significant differences between treatments in the brackish marsh ($P<0.05$, Tukey's post hoc test).

Metric	WO Mean ± S.E. (N)	WN Mean ± S.E. (N)	UC Mean ± S.E. (N)	F-value	P-value
Height (m/plot)	10.8 ± 3.8 (8)	6.8 ± 3.2 (4)	11.9 ± 1.26 (5)	0.43	0.793
Tiller (mm/plot)	24.6 ± 3.6 (8)	23.2 ± 5.44 (4)	45.5 ± 5.2 (4)	6.24	0.5651
No. Leaves/ plot	25.1 ± 4.16 (8)	19.0 ± 4.7 (4)	38.4 ± 5.4 (5)	3.53	0.611
Leaf Area (cm^2/plot)	737 ± 239 (8)	837 ± 276 (4)	2741 ± 477 (5)	0.047	0.957
Biomass (g/plot)	20.8^{a} ± 7.5 (7)	42.9^{b} ± 16.4(4)	164^{b} ± 24 (5)	23.22	**0.0028**
No. Plants/plot	2.9 ± 2.8 (8)	1.8 ± 1.8 (3)	2.1 ± 0.2 (5)	1.88	0.280
% Nitrogen	1.1 ± 0.1 (3)	0.85 ± 0.16 (3)	0.85 ± 0.02 (5)	3.49	0.0813
% Carbon	43.7 ± 0.3 (3)	44.9 ± 0.9 (3)	44.8 ± 0.3 (5)	1.34	0.314
% Sulfur	0.25^{a} ± 0.04 (3)	0.18^{ab} ± 0.02 (3)	0.13^{b} ± 0.01 (5)	6.65	**0.0199**
C:N	44.8 ± 4.3 (3)	66.3 ± 11.59 (3)	61.7 ± 1.76 (5)	3.23	0.093
N:S	10.8 ± 0.6 (3)	10.9 ± 0.9 (3)	15.4 ± 1.8 (5)	2.87	0.115

Table 2.7. Results of t-tests comparing growth parameters and tissue nutrient concentrations of *S. alterniflora* (SA) and *S. cynosuroides* (SC) in the salt marsh and brackish marsh environments either with or without neighbors. Differences between species within an environment by neighbor treatment are indicated in boldface.

Metric	Salt Marsh		Brackish Marsh	
	Without N	**With N**	**Without N**	**With N**
Height (m/plot)	**0.0072**	0.3377	0.0975	0.3118
Tiller (mm/plot)	0.3094	0.6099	**0.0402**	0.1410
No. Leaves/ plot	0.2731	0.1986	**0.0249**	na
Leaf Area (cm^2/plot)	**0.0099**	0.1643	0.2241	na
Biomass (g/plot)	**0.0013**	0.7662	0.5217	na
No. Plants/plot	**0.0152**	0.1372	0.1116	0.4425
% Nitrogen	na	**0.0133**	0.2093	0.1699
% Carbon	na	0.1170	0.288	na
% Sulfur	0.5789	**0.0138**	**0.0462**	**0.0350**
C:N	na	**0.0122**	0.1129	na
N:S	0.6987	0.4283	0.1699	0.2807

Table 2.8. *Spartina alterniflora* and *S. cynosuroides* reported elemental composition (% for N and S, ppm for all other nutrients). *S. alterniflora* values reported are for "tall" form plants unless otherwise noted. *S. cynosuroides* values are only available for % N, K, Na, and P (noted with SC in text).

Source	N	S	Al	Ba	Ca	Fe	K	Mg	Mn	Na	P	Si	Sr	Zn
Broome et al. (1975) NC	0.77-0.98	0.4-0.55	--	--	3200	701.6	7700	2900-4600	30.6	25000	1000-1200	--	--	--
Gallagher 1975 (GA)	0.7	--	--	--	2100	--	6300	4300	55	--	1400	--	24	13
Patrick & Delaune 1976	0.7-0.9	--	--	--	--	--	--	--	--	--	800-1500	--	--	--
Chalmers 1979 (GA)	1.4	--	--	--	--	--	--	--	--	--	--	--	--	--
Linthurst 1979	0.78-0.88	0.21-0.49	--	--	1500-3000	--	10200-14300	2400-3000	--	--	1100-1200	--	--	~20
Mendelssohn 1979	1.69	--	--	--	--	--	--	--	--	--	--	--	--	--
Gallagher et al. 1980	1.0	--	--	--	1500	--	10000	3300	--	--	1700	--	--	--
Smart & Barko[a] 1980	0.66-0.80	--	--	--	--	--	5700 (marine) 6100 (fresh)	--	--	7700 (marine) 7000 (fresh)	400-500	--	--	--
Linthurst & Seneca 1981	1.07	0.35	--	--	3000	--	12800	3500	--	--	2000	--	--	--
Carlson & Forrest 1982	--	0.13	--	--	--	--	--	--	--	--	--	--	--	--
Hopkinson & Schubauer 1983	1.05	--	--	--	--	--	--	--	--	--	--	--	--	--
Hopkinson & Schubauer 1984	~0.66 (SC)	--	--	--	--	--	--	--	--	--	--	--	--	--
Valiela 1984	1.4	--	--	--	--	--	--	--	--	--	--	--	--	--
Broome et al. 1986	0.7	0.5	--	--	2500	--	7800	4600	--	900		--	--	--
Hackney & de la Cruz 1986 (MS)	~0.4-1.0 (SC)	--	--	--	--	--	--	--	--	700 (SC)	--	--	--	--
Ornes & Kaplan 1989 (GA)	1.32	0.32	--	--	2600	--	12300	3500	--	--	1900	--	--	--

Source	N	S	Al	Ba	Ca	Fe	K	Mg	Mn	Na	P	Si	Sr	Zn
Beale & Long 1991 (United Kingdom)	0.3 (SC)	--	--	--	--	--	1000 (SC)	--	--	--	400 (SC)	--	--	--
Bradley & Morris[b] 1991	--	0.32 0.35	--	--	2004 1603	--	14076 8602	4132.7 8508.5	--	45520 117708	--	--	--	--
Stribling & Cornwell 2001 (MD)	~0.75-3.07 (freshwater) ~0.05-0.2 (saltwater)	~0.33-0.53 (freshwater) ~0.24-0.69 (saltwater)	--	--	--	--	--	--	--	300-3800 (freshwater) 500-2000 (saltwater)	--	--	--	--
Ornes et al 1998	--	--	~800-4000	--	--	320-870	--	--	25-80	--	--	--	--	8-16
DeLaune et al 1983	--	--	--	--	--	--	--	--	--	--	--	--	--	13

[a] Critical nutrient levels of N and P.

[b] Values for plants grown in salinity treatments of 10 and 40 g/dm^3 respectively.

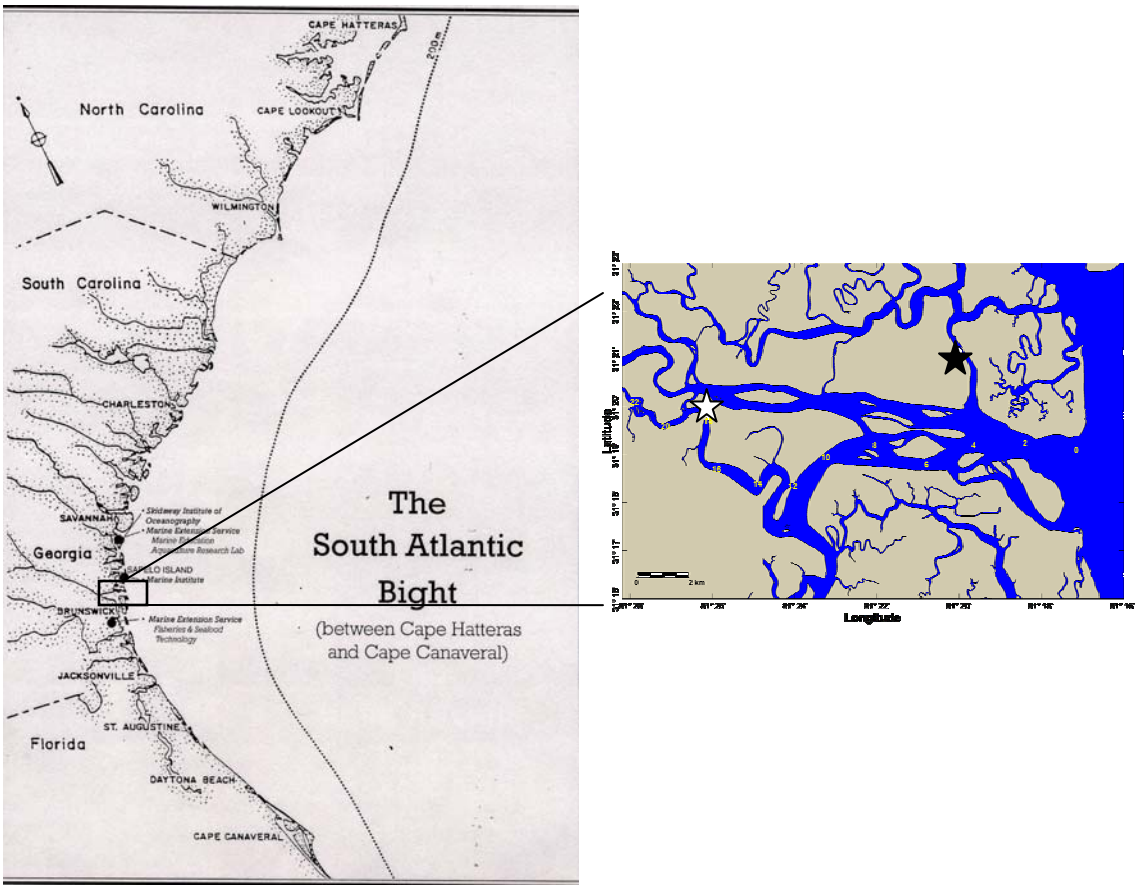

Figure 2.1. Location of reciprocal transplant marshes along the Altamaha River estuary, GA. The white star indicates the *S. cynosuroides* transplant habitat and the black star indicates the location of the *S. alterniflora* transplant habitat.

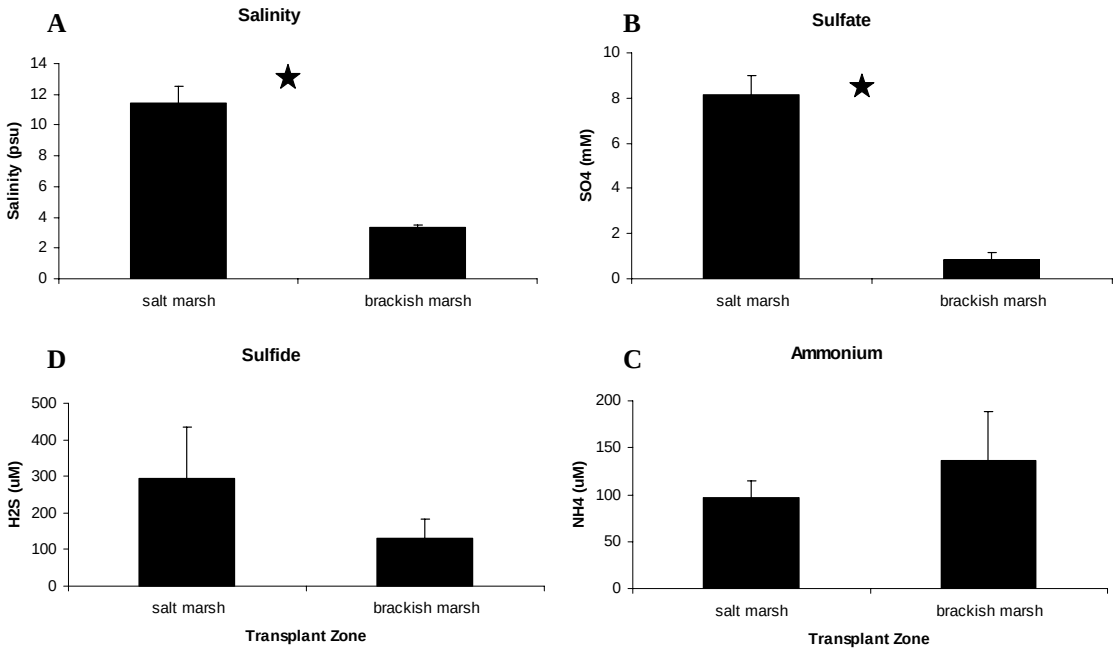

Figure 2.2. Abiotic parameters in the salt and brackish marsh environments. Data represent marsh environmental parameters compiled means from all sample dates in each transplant environment. Error bars represent standard errors. Stars indicate differences ($P < 0.05$, ANOVA) between parameters in the salt marsh as compared to the brackish marsh environment.

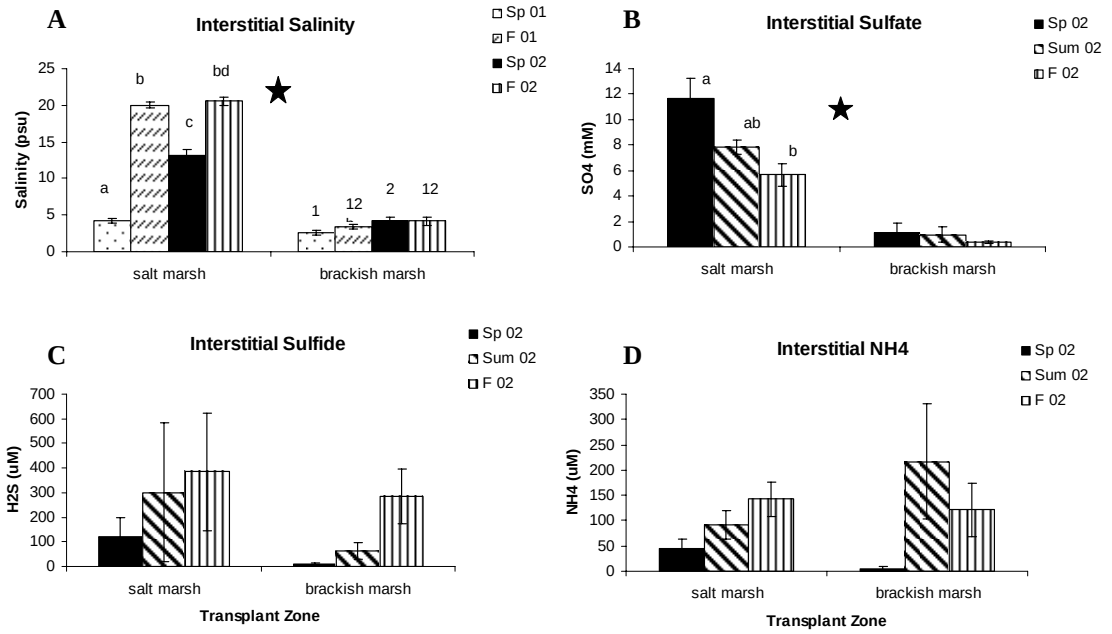

Figure 2.3. Abiotic parameters in the salt marsh and brackish marsh environments. Data represent marsh environmental parameters over one or two growing seasons. Error bars represent standard errors. The data are means (± SE) of the sample date in each transplant environment. Stars indicate differences ($P < 0.05$, ANOVA) between parameters in either the salt marsh or the brackish marsh environment. Different letters indicate significant differences between parameters in the salt marsh environment and different numbers indicate significant differences between parameters in the brackish marsh environment ($P < 0.05$, Tukey's post hoc test).

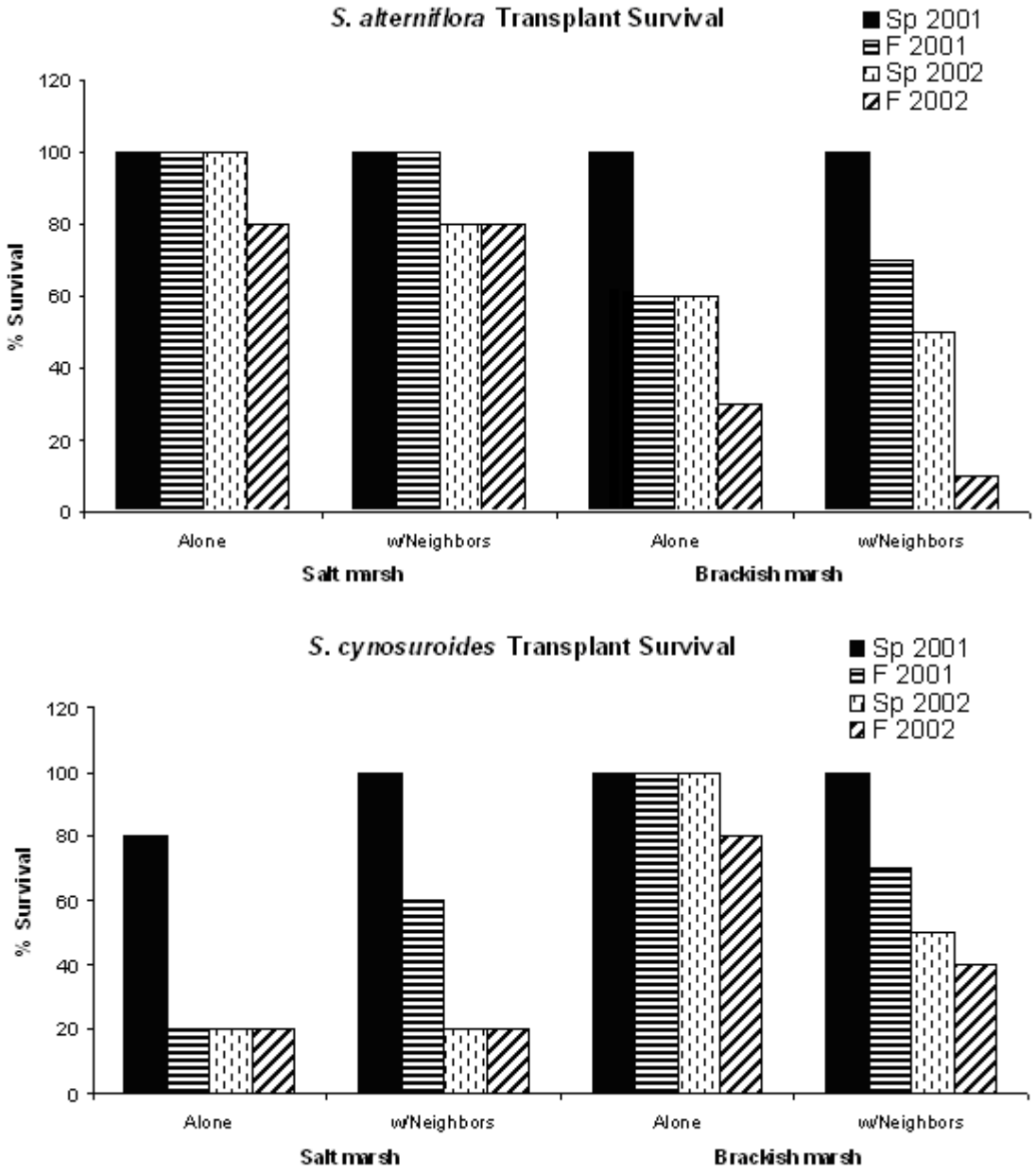

Figure 2.4. Percent survival of reciprocal transplants of *S. alterniflora* (top) and *S. cynosuroides* (bottom) over the course of two growing seasons in salt and brackish marsh environments, either alone or with neighbors present.

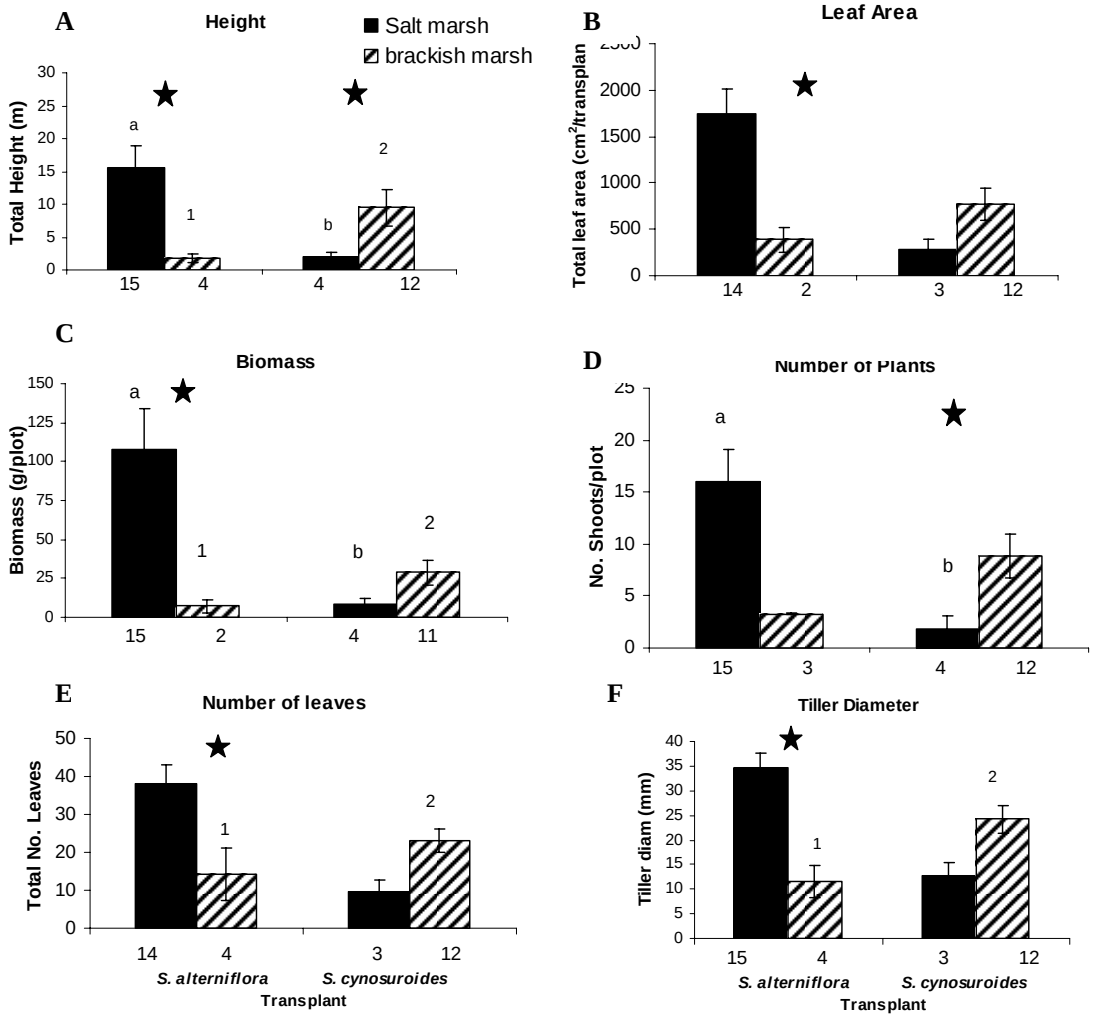

Figure 2.5. Results of reciprocal transplant experiments where monocultures of *S. alterniflora* and *S. cynosuroides* were transplanted into either a salt marsh (solid bar) or brackish marsh (notched bar) environment. Data represent transplant characteristics after two growing seasons, regardless of the presence or absence of neighbors. Error bars represent standard errors. Numbers located beneath each bar represent N. Characteristics reported for each species includes: A-height (m/plot), B-total leaf area (cm^2/plot), C-biomass (g/plot), D-number of plants (no./plot), E-number of leaves (no./plot), and F-tiller diameter (mm/plot). Stars indicate differences between environments within a species (i.e. *S. alterniflora* height is greater in the salt marsh than in the brackish marsh; $P < 0.05$, ANOVA). Different letters indicate significant differences between species in the salt marsh environment and different numbers indicate significant differences between species in the brackish marsh environment ($P < 0.05$, Tukey's post hoc test).

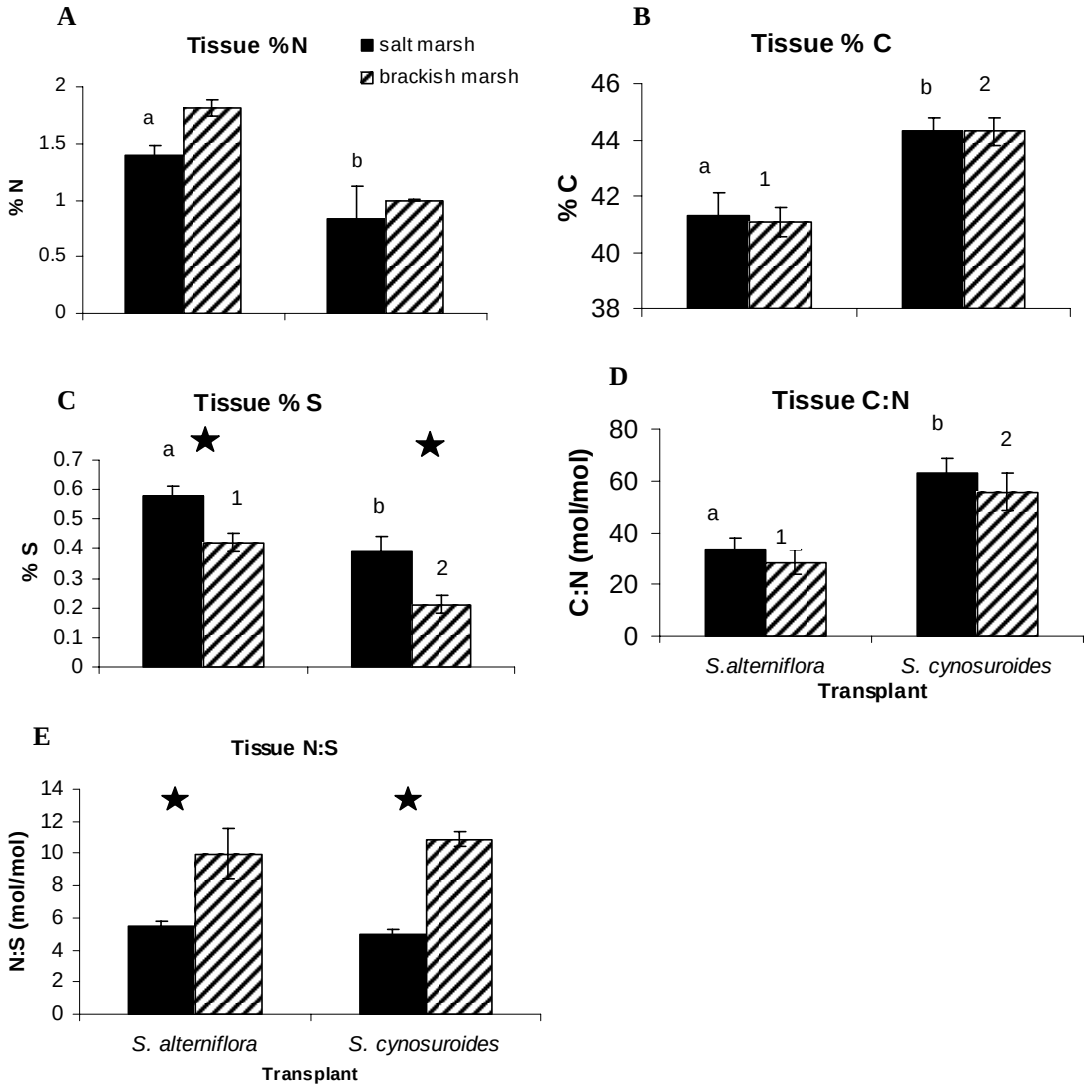

Figure 2.6. Influences of transplant environmental conditions on final transplant tissue nutrient concentrations after two growing seasons, regardless of the presence or absence of neighbors, in the salt marsh (solid bar) and brackish marsh (notched bar). Error bars represent standard error (*S. alterniflora* N=7 and 4 in the salt and brackish marsh respectively; *S. cynosuroides* N=11 and 6 in the salt and brackish marsh respectively). Characteristics reported include: *A*- % nitrogen, *B*- % carbon, and *C*- % sulfur, *D*- carbon:nitrogen ratio (mol/mol), and *E*-nitrogen:sulfur ratio (mol/mol) (by weight). Stars indicate differences between environments within a species (i.e. *S. alterniflora* % N is greater in the brackish marsh than in the salt marsh; $P < 0.05$, ANOVA). Different letters indicate significant differences between species in the salt marsh environment and different numbers indicate significant differences between species in the brackish marsh environment ($P < 0.05$, Tukey's post hoc test).

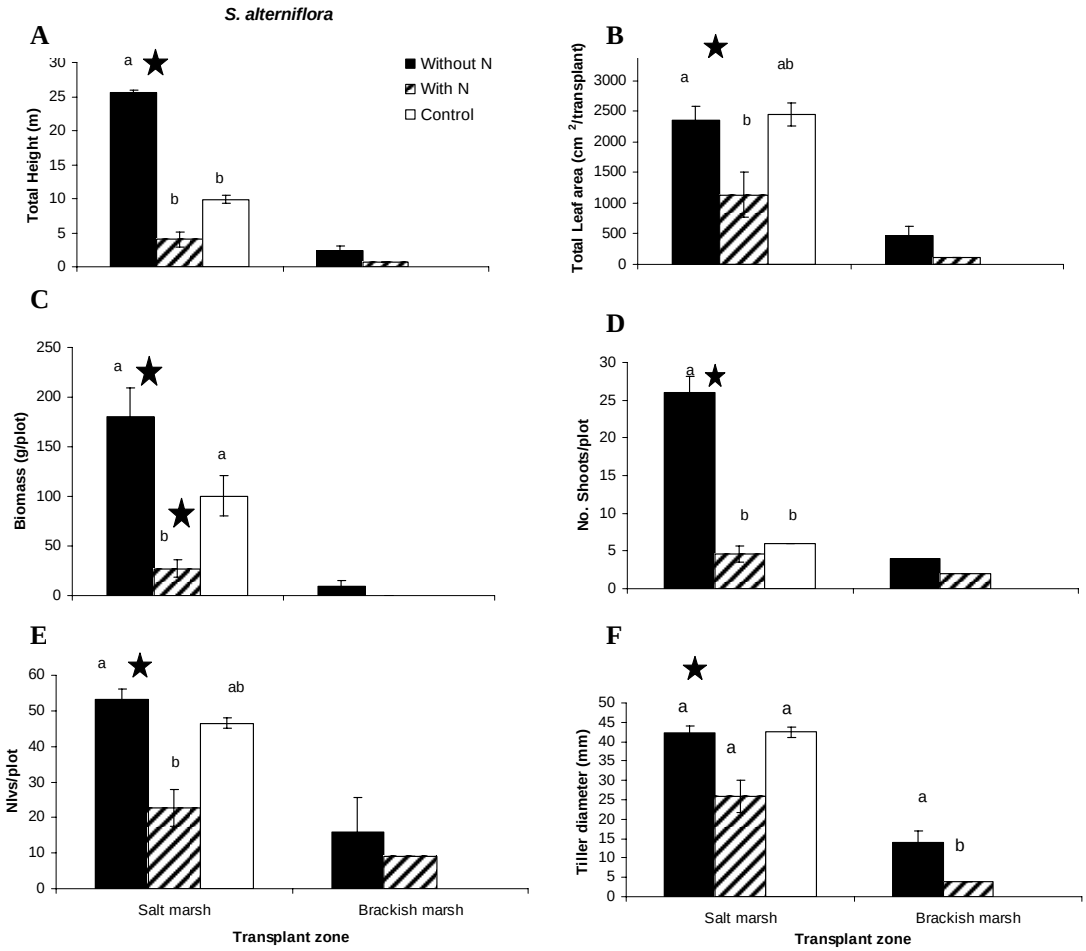

Figure 2.7. Results of *S. alterniflora* reciprocal transplants into either a salt marsh (solid bar) or brackish marsh (striped bar) environment with surrounding neighboring vegetation removed (without N) or present (with N) after two growing seasons. Transplant controls (control; N=4) were 1 m^2 plots of unmanipulated vegetation near the transplant sites. The data are means (±SE, N=7-8 plants in the salt marsh and N=1 or 3 plants in the brackish marsh) for *S. alterniflora* in each transplant environment for *A*-height (m/plot), *B*-total leaf area (cm^2/plot), *C*-biomass (g/plot), *D*-number of plants (no/plot), *E*-number of leaves (no./plot), and *F*-tiller diameter (mm/plot). Stars indicate differences ($P < 0.05$, ANOVA) within a neighbor treatment between the salt and brackish marsh environment. Different letters indicate significant differences among neighbor treatments in either the salt or brackish marsh environment ($P < 0.05$, Tukey's post hoc test).

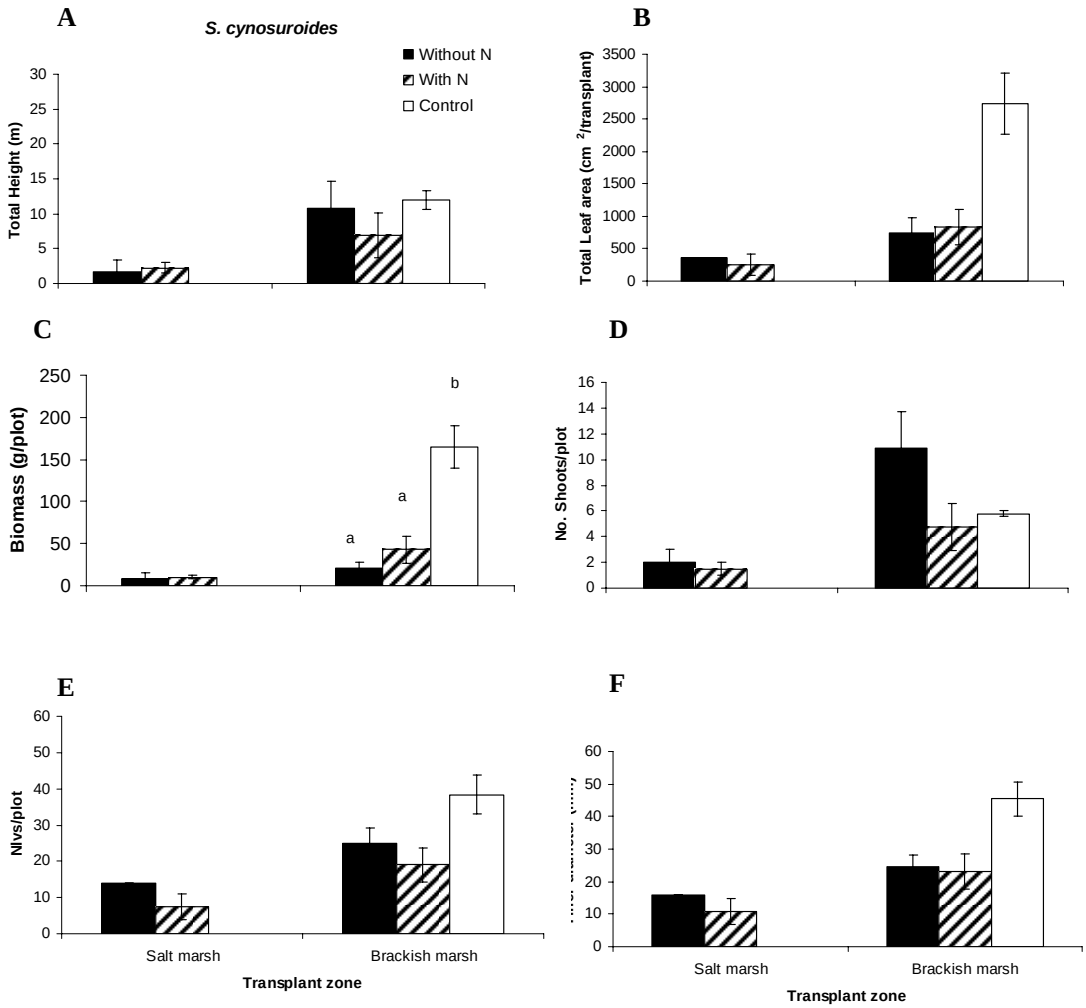

Figure 2.8. Results of *S. cynosuroides* reciprocal transplants into either a salt marsh (solid bar) or brackish marsh (striped bar) environment with surrounding neighboring vegetation removed (without N) or present (with N) after two growing seasons. Transplant controls (control; N=4) were 1 m^2 plots of unmanipulated vegetation near the transplant sites. The data are means (± SE, N=1-2 plants for salt marsh and N=7-8 plants for brackish marsh) for *S. cynosuroides* in each transplant environment for *A*-height (m/plot), *B*-total leaf area (cm^2/plot), *C*-biomass (g/plot), *D*-number of plants (no./plot), *E*-number of leaves (no./plot), and *F*-tiller diameter (mm). Different letters indicate significant differences among neighbor treatments in either the salt or brackish marsh environment ($P < 0.05$, Tukey's post hoc test).

CHAPTER 3

SPARTINA ALTERNIFLORA AND *S. CYNOSUROIDES* GROWTH RESPONSES UNDER MANIPULATED ABIOTIC AND BIOTIC CONDITIONS

Creekbank vegetation along the Altamaha River estuary, GA is dominated by two *Spartina* species, *S. alterniflora* Loisel (smooth cordgrass) and *S. cynosuroides* (L.) Roth (big cordgrass). *Spartina alterniflora* forms dense, monospecific stands in high salinity marshes (typically > 15 psu) closest to the mouth of the river; mixed communities of both species are found in the mesohaline reaches (~1.5 – 15 psu) and *S. cynosuroides* is found in monospecific stands in the upper brackish, or oligohaline (typically < 1.5 psu) sections of the river (Pomeroy and Wiegert 1981, Eleuterius 1990, White 2004). Little attention has been directed towards understanding the mechanisms that control marsh plant distributions along the longitudinal axes of estuaries, such as exists along the Altamaha River estuary. However, the mechanisms that maintain plant zonation in a single marsh are not necessarily the same as those that structure creekbank distributions along an estuarine salinity gradient.

Much of our understanding of the processes that control the distribution of marsh vegetation is based on research in salt marsh communities, where the focus is on the elevation gradient from the creekbank, through the inland marsh to the upland border. The view that salinity is the major determinant of vegetation patterns in the salt marsh is too simplified (Odum 1988). Multiple abiotic and biotic influences, often intricately

linked and acting concurrently within the marsh system, more completely explain vegetation patterns than any single environmental parameter. Although salinity is certainly a dominant abiotic constraint, a plant's physiological response to nutrient availability (e.g. nitrogen, phosphorous or sulfate), tidal inundation, and microscale soil conditions can be equally influential in creating zonation patterns (Howes et al. 1981, Mendelssohn et al. 1981, Pomeroy and Wiegert 1981, King et al. 1982, DeLaune et al. 1983, Bertness and Ellison 1987, Mendelssohn and McKee 1988, Bertness 1991b, Pezeshki and Delaune 1991, Mitsch and Gosselink 1993, Pezeshki and Delaune 1995). In addition to these abiotic conditions, biotic influences such as plant competition and/or facilitation interactions must be considered as other mechanisms defining vegetation patterns in the salt marsh. A typical United States east coast salt marsh is characterized by *S. alterniflora* dominating creekbank environments due to its physiological tolerance of harsh abiotic conditions such as high salinity and tidal inundation (Bertness and Ellison 1987, Bertness 1991b, Shumway and Bertness 1994). *Spartina alterniflora* is also confined to the creekbank due to biotic interactions as this plant is competitively inferior at mid-marsh and upland habitats where more competitive but less physiologically hardy species such as *Juncus gerardi* (or *J. roemerianus*), *Distichlis spicata*, *Borrichia frutescens* and *Iva frutescens* dominate (Bertness 1991a, Levine et al. 1998, Hacker and Bertness 1999). Generally, the upper elevation distributions of a species in the marsh is limited by competitive interactions, thus poorly competing species are displaced to lower tidal elevations (Bertness 1991b, Pennings and Callaway 1992, Levine et al. 1998, Hacker and Bertness 1999). It is also possible for facilitative interactions between species to buffer harsh environmental conditions and influence plant

distribution along the elevation gradient in a marsh (Bertness et al. 1992, Bertness and Shumway 1993, Bertness and Yeh 1994, Bertness and Hacker 1994, Hacker and Gaines 1997).

Given the complexity of zonation controls observed in salt marshes, it is likely that a combination of abiotic and biotic mechanisms are responsible for the observable vegetation patterns along estuaries as well. Latham et al. (1991) found that the distribution of *Scirpus validus* in brackish and freshwater marshes on the Savannah River, GA changed with salinity, soil organic matter and elevation. Higinbotham et al. (2004) used GIS and aerial photographs to identify broad vegetation classes and their distribution along the Altamaha and Satilla Rivers in coastal Georgia. They suggested that the distribution of their four vegetation categories (salt marsh, brackish marsh, Juncus marsh, and fresh marsh) was driven primarily by estuarine salinity ranges. Greenhouse experiments by Stribling (1997) suggested that *S. alterniflora* distribution in oligohaline marshes in Maryland could be limited by sulfate supply as it was shown that this species might have unusually high sulfate requirements.

In Chapter 2 I conducted a reciprocal transplant experiment in an attempt to explicitly determine whether the controls described for a single salt marsh, namely physiological tolerance to environmental conditions and competition (with and without neighbors), could describe *Spartina* plant distribution patterns along the Altamaha River estuarine gradient in Georgia. Results from this experiment suggested that the distribution of *Spartina* species was influenced by a complex combination of abiotic and biotic factors. *Spartina cynosuroides* did poorly in the salt marsh, suggesting that its lower distribution is controlled by environmental conditions (most likely high salinity).

This result is expected given the species' natural brackish marsh distribution. Interestingly, *S. alterniflora* exhibited poor transplant survival in the brackish environment (regardless of neighbor presence) suggesting that it too is controlled by environmental conditions along the estuary. It was not clear why *S. alterniflora* performed poorly in the brackish marsh as neither plant tissue % S nor % N values were below previously reported ranges where *S. alterniflora* exhibited successful growth (in either brackish or salt marsh environments (Smart and Barko 1980, Ornes and Kaplan 1989, Stribling and Cornwell 2001). However, N:S ratios of *S. alterniflora* transplants were greater in the brackish marsh as compared to values found for transplants in the salt marsh, suggesting that sulfur was less available in the brackish marsh. These N:S results are similar to those found by Stribling (1994) and indicate that the lower sulfate concentrations in brackish marshes may play a role in limiting *S. alterniflora*'s upper estuarine distribution.

Greenhouse experiments are useful tools for investigating specific growth conditions for marsh plants under controlled environments, and often this information can be extrapolated to help explain field distributions. Howard and Mendelssohn (1999) investigated the effects of increasing salinity on four oligohaline plant species in a greenhouse experiment and observed that salinity tolerances in the greenhouse reflected the distribution of these species in coastal habitats of the Gulf of Mexico. Manipulative greenhouse experiments also identified what type of soil and inundation conditions would favor *Phragmites australis* success and expansion in an African estuary (Adams and Bate 1999). Stribling (1997) first identified the possibility that *S. alterniflora* distribution might be constrained by low sulfate conditions in the field through greenhouse

experiments. In these experiments she demonstrated that *S. alterniflora* growth indices (i.e. total number of leaves, total leaf length, and relative growth rates) responded positively to increasing sulfate concentration, whereas those of *S. cynosuroides* did not.

In order to better define potential mechanisms controlling *Spartina* distribution along the Altamaha River estuary, I designed a series of greenhouse experiments to investigate the growth response of both *S. alterniflora* and *S. cynosuroides* to field ranges of salinity and sulfate concentrations as well as to explicitly address the influence of neighbor presence on these results. If salinity is the main factor that controls *Spartina* distribution along an estuarine gradient, then we would expect to see decreased *S. cynosuroides* growth at high salinities and increased *S. alterniflora* growth at lower salinities. If there is some type of sulfate nutrient requirement for *S. alterniflora*, we would expect to see reduced growth of this species in treatments that had low sulfate additions whereas *S. cynosuroides* would not respond to changes in sulfate concentrations. If *S. alterniflora* is confined to the lower estuary by competitive interactions, we would expect to see facilitative interactions in the harsher, high salt treatments and competitive interactions in the more benign, low salt treatments. In this chapter, I describe *Spartina* survival, growth and sediment conditions in greenhouse manipulations and attempt to integrate information on plant physiological tolerances, plant-plant interactions and nutrient requirements from greenhouse and field experiments into a general explanation of the mechanisms that generate the creekbank vegetation patterns observed along the Altamaha River.

MATERIALS AND METHODS

Spartina Transplants

Small sections (approximately 25 x 25 cm in width and 25 cm deep) of riverbank marsh soil with associated *S. alterniflora* or *S. cynosuroides* shoots (approximately 20 cm in height) were excavated with shovels from monotypic stands in each of the species' natural salinity ranges along the Altamaha River in March 2002 (Experiment 1) and May 2002 (Experiment 2). The sods were transported back to greenhouses at the University of Georgia, placed in shallow bins, and vegetatively propagated under freshwater conditions until appropriate numbers of new shoots were available for experiments. This propagation in the greenhouse reduced any field-induced ecophenic variation associated with the plants (Hester et al. 1998). New shoots (mean height approx. 25 cm) were separated from the parent ramet, washed free of substrate (sand and marsh mud) and each plant was placed into a 12 x 12 cm pot with a 25:75 mixture of peat moss and sand.

Experimental Design

The first experiment investigated the response of both *S. alterniflora* and *S. cynosuroides* to combined salinity and sulfate treatments. Plants were grown in a temperature and light-controlled greenhouse with a photoperiod of 14 hours. Daytime temperatures were maintained at approximately ~30 °C and night temperatures at approximately ~25 °C. Three salinity levels (NaCl; measured in practical salinity units, or, psu) and three sulfate levels ($NaSO_4^{-2}$; measured in mM) were applied in 8 treatment combinations as follows: 0 psu:0 mM SO_4^{-2}, 0 psu:4 mM SO_4^{-2}, 5 psu:0 mM SO_4^{-2}, 5 psu:4 mM SO_4^{-2}, 5 psu:28 mM SO_4^{-2}. 35 psu:0 mM SO_4^{-2}, 35 psu:4 mM SO_4^{-2}, and 35

psu:28 mM SO_4^{-2} (Figure 3.1). Tap water was used in the 0 psu: 0 mM SO_4^{-2} treatment and mixed with sodium sulfate and sodium chloride to create the described treatment combinations. There were 7 replicate pots for each treatment combination. Treatments were maintained from June 2002 to November 2002. It should be noted that one potential combination (0 psu: 28 mM SO_4^{-2}) was not applied during this experiment as the ionic strength of this combination was too similar to treatments at the 5 psu level ($I = \frac{1}{2} \Sigma mz^2$; m=molality and z= ion charge, Figure 3.1) which would confound our ability to differentiate treatment effects (either salinity or sulfate) from the more general impact of ionic strength effects.

The second experiment examined the impact of salinity and sulfate treatments in addition to the presence or absence of neighbors on *Spartina* performance. Plants were grown in a naturally lighted greenhouse (temperature ~ 25 °C). Two NaCl levels (0 and 35 psu), two SO_4^{-2} levels (0 and 28 mM), and three neighbor treatments (target species alone, with the opposite species, or with conspecifics) were applied in 12 treatment combinations for each *Spartina* species (Figure 3.1). There were 4 replicate pots for each treatment combination. Neighbor treatments consisted of one target plant and three neighbors placed in a triangular fashion around the target, equidistant from the target and the pot edges. Treatments were maintained from August 2002 to December 2002.

In order to avoid any treatment shock associated with high ionic strengths at the 35 psu levels, salinities were gradually increased from 1 psu to 35 psu in both experiments over the course of 3 weeks. Sulfate concentrations were not gradually ramped up to their final target concentrations as ionic strength concerns focused on the larger effects of NaCl. After reaching target salinities, appropriate NaCl: SO_4^{-2}

treatments were applied 3 times per week. Plants were saturated with treatments (~1 L/pot) such that excess liquid drained out of the pots. Pilot experiments indicated that this regime was sufficient for maintaining appropriate concentrations of NaCl and SO_4^{-2} in each treatment. Plants were moderately watered between treatments to eliminate salt buildup within the pots and thus help maintain appropriate treatment concentrations. UGA Plant Biology Greenhouse staff watered daily and fertilized all pots weekly with Peter's Pete-Lite-Special (~ 0.5 L/pot of fertilizer with 300 ppm N: 150 ppm P: 300 pmm K) (Table 3.1). The sulfur present in the fertilizer (~1.48 mg S added per week), was minimal in contrast to the treatments: the 4 mM treatment had ~128.24 mg S added three times/week and the 28 mM S treatment had ~ 897.68 mg S added three times/week. Moreover, interstitial nutrients concentrations in the freshwater treatments were below the "critical level" for *S. alterniflora* growth (0.5-1.0 mM) as reported by Stribling (1994). Thus the fertilizer did not affect our ability to distinguish among sulfate treatments (0 mM, 4 mM, and 28 mM).

Characters Measured

Non-destructive measurements of plant performance were monitored at the beginning and end of each experiment. Measurements included the total number of shoots and seedheads, plant height and tiller diameter (of 10 randomly selected plants), and average photosynthetic leaf area of 5 plants (3 randomly selected leaves had both leaf lengths and widths measured on each of the 5 plants) in each pot. Plants were harvested at the end of the experiment. Aboveground material was clipped just above the soil surface and both above- and belowground material was washed free of debris. Target and neighbor plants were separated, rinsed with deionized water, and dried at 60º C to a

constant weight. Only the aboveground target plant material (stems and leaves), including those plants connected to the target by rhizomes, was ground in a Wiley Mill (40 µm mesh) for elemental nutrient analysis. Carbon, nitrogen, and sulfur concentrations of aboveground plant tissue were analyzed with a CE Elantech Flash Elemental Analyzer 1112. Ground plant tissues were not acidified before CNS analysis as preliminary runs did not show significant differences in C, N or S between acidified and un-acidified samples. Acid extracts (Sah and Miller 1992) of selected greenhouse plant samples were analyzed for Al, Ba, Ca, Fe, K, Mg, Mn, Na, P, Si, Sr and Zn on an inductively coupled plasma-atomic emission spectrophotometer (University of Georgia Chemical Analysis Lab). Plant samples from naturally occurring *S. alterniflora* and *S. cynosuroides* stands as well as samples from a separate reciprocal transplant experiment (see Chapter 2) were analyzed for these same nutrients in order to compare greenhouse values with observations from plants growing in the field.

We quantified interstitial pore water salinity, ammonium, nitrate + nitrite, sulfate, and sulfide concentrations at the beginning and end of each experiment. We took 3 replicate samples from all 8 treatment combinations for each *Spartina* species in the first experiment whereas in the second experiment we took 3 replicate samples from all 12 treatment combinations for each *Spartina* species. Water for these analyses was collected in dishes placed beneath the pots after treatments were applied. Dissolved free sulfide was determined from water (10 ml) drawn directly from the collection dish, filtered through a 0.2 µm Gelman Acrodisc, and fixed in the greenhouse in acid washed scintillation vials containing 0.05 M zinc acetate (Cline 1969). The remaining water (ranging from 10-120 ml) was collected in acid-washed containers, stored in a cooler, and

transported back to the laboratory. This sample was immediately filtered (pre-combusted Whatman GF/F, 47 µm filter as well as through 0.2 µm Gelman Acrodisc filters) and interstitial salinity was measured with a portable refractometer (Leica Model 10419, automatically temperature compensated). The remaining sample was divided into aliquots for subsequent ammonium and sulfate analyses. Ammonium samples were stored frozen prior to analysis whereas sulfate and sulfide samples were refrigerated. Sulfate samples were acidified with 100 µL of 70 % HNO3 prior to freezing. Ammonium concentrations were determined using colorimetric analysis and measured spectrophotometrically (Koroleff 1983; Shimadzu UV-1601). Sulfate and sulfide were analyzed by M. Erickson and B. Porubsky in M. Joye's laboratory at the University of Georgia. Sulfate was analyzed by ion chromatography (EPA Method 300.1, Dionex LC20) and sulfide was measured spectrophotometrically (Cline 1969, Shimadzu UV-1601).

Data Analysis

In both experiments, data were analyzed using analysis of variance (ANOVA) with species and treatment as fixed effects (Sokal and Rohlf 1995, SAS 2000). Initial measures were used as a standard covariate in all cases. A significance level (alpha) of 0.05 was used for all statistical analyses unless otherwise stated. All data were tested for normality (Shapiro-Wilk test statistic) and homogeneity of variance (Bartlett test statistic) prior to analysis. Where necessary, data were appropriately transformed to meet these assumptions. We ran ANOVA models that incorporated the effects of species, treatment, sodium, sulfate (and neighbor in the second greenhouse experiment). Separate models were also run for each species. Tukey's post-hoc tests were used to look at the effect of

species and treatment on plant performance and edaphic parameters. T-tests were used to test for differences between initial and final ammonium values. Plant mortality is a nominal response variable (dead or alive) and logistic analyses were used to assess significance of zone and treatment on *Spartina* survival (SAS Institute 2000).

RESULTS

Greenhouse Experiment 1

This component of the research explored *S. alterniflora* and *S. cynosuroides* responses to different concentrations of salinity and sulfate.

Environmental Growth Conditions

Interstitial waters were sampled at the beginning (June) and end (November) of the experiment in pots containing both *Spartina* species to characterize water quality conditions. Plant species did not influence soil nutrient concentrations, so data from both species were averaged within treatments (Table 3.2).

Salinity and sulfate concentrations generally reflected the experimental treatments administered to the plants. Salinities from the beginning and end of the experiment averaged 0.04 ± 0.04 psu in the freshwater treatments, 4.14 ± 0.25 psu in pots that were treated with 5 psu, and 22.46 ± 0.78 psu in pots treated with 35 psu ($F=622.85$, $p<0.0001$). Sulfate concentrations from the beginning and end of the experiment averaged 0.68 ± 0.15 mM in 0 SO_4^{-2} treatments, 4.16 ± 1.23 mM in 4 SO_4^{-2} treatments, and 13.38 ± 1.47 mM in treatments with 28 mM SO_4^{-2} additions ($F=33.17$, $p<0.0001$). Both the high salinity and high sulfate treatments fell below the target treatment

concentrations but were still significantly greater than the lower two treatment concentrations. Final ionic strengths calculated for all treatment combinations were lower than target values (Table 3.3). Assuming that ionic strengths greater than full-strength seawater can be detrimental for plant growth, our low values suggest that all the plants were grown under low ionic stress conditions.

Initial and final sulfide values did not differ, so these values were also averaged (Table 3.2). Overall, sulfide concentrations were low (below detection in the high salinity treatments) suggesting that the soils were well flushed and the soil environment was well oxidized.

Initial and final ammonium concentrations for each treatment are reported separately, as these concentrations changed over time (Table 3.2). Final ammonium values were all greater than initial values and increased with increasing salinity ($F=151.99$, $p<0.0001$) but not with increasing sulfate concentration ($F=1.47$, $p=0.2446$). Final ammonium concentrations showed extremely large increases in all but the three lowest ionic strength treatments (0 psu: 0 mM SO_4^{-2}, 0 psu: 4 mM SO_4^{-2}, and 5 psu: 0 mM SO_4^{-2}), and it may be that these higher values resulted from competitive inhibition of nitrogen uptake by greater NaCl presence. Initial concentrations of ammonium ranged from ~5-52 μM, whereas final concentrations were often 100-fold higher. The large ammonium concentrations are well above previously reported values for *S. alterniflora* mashes (Mendelssohn 1979, Stribling and Cornwell 2001) and may have inhibited *Spartina* growth over the course of this experiment. Redox potentials were not measured in this experiment, however, it would appear unlikely that these ammonium conditions negatively influenced the oxidation status of the soils as the sulfide concentrations were

all very low. Measurements of pH were not taken over the course of this experiment. It is possible that the pH of the soils fell outside the average marsh pH (6-6.5) due to the high ammonium concentrations.

Effects of Treatments on *Spartina* Performance

Transplant shoot height of both species was approximately 0.25 cm, however, when initial heights were measured 3 weeks after planting (once treatment concentrations were established), *S. cynosuroides* shoots were significantly taller than *S. alterniflora* shoots. *Spartina cynosuroides* averaged 0.63 ± 0.03 m and *S. alterniflora* averaged 0.37 ± 0.01 m ($p<0.0001$). Other initial plant characteristics (i.e. tiller diameter, number of leaves and leaf area) did not differ between species.

Survival

Spartina cynosuroides survival was 100 % in all but the 0 psu: 4 mM treatment, where it was 86 % (Figure 3.2). In contrast, *S. alterniflora* survival was 100 % in all 4 treatments with low ionic strength (0 psu: 0 mM SO_4^{-2}, 0 psu: 4 mM SO_4^{-2}, 5 psu: 0 mM SO_4^{-2}, and 5 psu: 4 mM SO_4^{-2}) and extremely poor in the remaining half of the treatments (14 % in 5 psu: 28 mM SO_4^{-2} and 35 psu: 0 mM SO_4^{-2} and 0% in 35 psu: 4 mM SO_4^{-2} and 35 psu: 28 mM SO_4^{-2}). Mortality was assessed one month after the experiment began, and at that point only one transplant of each species had died; no plants were re-planted.

Plant growth parameters

Both *S. alterniflora* and *S. cynosuroides* showed similar decreases in growth with increasing salinity but little change in response to increasing sulfate concentrations

(Figures 3.3-3.6 and Tables 3.4-3.7). The *S. alterniflora* plants that did survive at these higher salinity treatments showed similar patterns to survival data, with comparable growth in the four low salinity and low sulfate combinations, reductions in growth at 5 psu: 28 mM SO_4^{-2}, and very poor growth at 35 psu: 0 mM SO_4^{-2}. This pattern is observable for plant height, leaf area, number of plants, and biomass measures (Figures 3.3 and 3.4). Biomass measures for *S. alterniflora* were the only parameters that showed significant reduction with increasing sulfate treatments (Table 3.5). Growth patterns of *S. cynosuroides* were similar to those observed for *S. alterniflora* even though *S. cynosuroides* survival was much higher: performance was best in the four low salinity and low sulfate combinations, reduced at 5 psu:28 mM SO_4^{-2} and poorest in the 35 psu treatments (Figures 3.5 and 3.6). All parameters showed a significant salinity effect, whereas only height and leaf area showed significant decreases with increasing sulfate treatment (Table 3.6).

There were some significant treatment effects on plant tissue nutrient concentrations. Increased salinity resulted in decreased tissue % C in *S. alterniflora*, whereas increased sulfate concentrations resulted in decreased % C but a significant increase in % S. However, we did not have any sample material from *S. alterniflora* at 35 psu so these observations are limited to only the 0 and 5 psu salinity treatments. *Spartina cynosuroides* had a significant decrease in % S with increasing salinity, which led to an increase in N:S ratios. There was no sulfate effect on *S. cynosuroides* tissue nutrients.

Greenhouse Experiment 2

This experiment explored *S. alterniflora* and *S. cynosuroides* survival and growth responses to different combinations of salinity and sulfate concentrations while at the same time manipulating their biotic environment through the presence or absence of neighbors.

Environmental Growth Conditions

Interstitial water was sampled from pots containing both *Spartina* species at the beginning (August) and end (December) of the experiment to characterize water quality conditions. *Spartina alterniflora* and *S. cynosuroides* target plant presence did not influence soil nutrient concentration, nor did neighbor identity/presence, thus data within each salinity and sulfate treatment are averaged regardless of species or neighbor presence (Table 3.8).

Analyses were similar to those from the first greenhouse experiment, where initial and final salinity and sulfate concentrations were averaged together and compared to target treatment concentrations. Interstitial sulfate and sulfide concentrations were not sampled in 0 psu: 0 mM SO_4^{-2} treatments nor in the 35 psu: 0 mM SO_4^{-2} treatments for two reasons. First, water nutrients sampled in greenhouse experiment 1 showed maintenance of treatment applications at these points; and second, we were interested in knowing more about how interstitial nutrients responded to high sulfate additions (0 psu: 28 mM SO_4^{-2} and 35 psu: 28 mM SO_4^{-2}). Final salinity and sulfate concentrations were close to target treatments in the low salinity and sulfate combinations (Table 3.8). However, in the high salinity and sulfate treatments, final concentrations were below target treatments. Salinities averaged 0.46 ± 0.12 psu in freshwater treatments and 15.18

± 0.51 psu in high salinity treatments. Sulfate concentrations averaged 0.42 ± 0.05 mM SO_4^{-2} in the 0 mM treatment and 8.73 ± 0.62 mM SO_4^{-2} in the treatment with 28 mM SO_4^{-2}. As in the first experiment, calculated ionic strengths in this experiment were again smaller than target values (Table 3.3) suggesting that these low ionic strengths had little negative influence on plant growth.

Initial and final sulfide values did not differ across treatments, so they were collapsed together. Whereas sulfide concentrations did not differ among treatments in the first greenhouse experiment, sulfide concentrations in this second experiment increased with increasing sodium and sulfate treatments ($p=0.0032$). Values were less than 25 µM, which is still lower than reported literature values for creekside marsh sites that generally range from ~ 40-223 µM (Morris et al. 1996, Baldwin and Mendelssohn 1998). This suggests that once again, soil conditions in the experiment were well oxidized and sulfide levels were not inhibiting *Spartina* growth.

In contrast to the first greenhouse experiment, initial ammonium concentrations ranged from 250-955 µM and were higher than final concentrations, particularly in the low salinity treatments (Table 3.8). Final values increased with increasing salinity level from an average of 4.58 ± 1.44 µM at 0 psu to 536.89 ± 46.93 µM at 35 psu. Final ammonium concentrations in treatments up to 0 psu: 28 mM SO_4^{-2} are within the range of reported values in salt marshes (Mendelssohn 1979, Craft et al. 1991, Stribling and Cornwell 2001), and there are additional reports of short form *S. alterniflora* marshes with ammonium concentrations up to ~242 µM (Mendelssohn 1979). These ammonium concentrations are considerably lower than those observed in the first greenhouse experiment.

Effects of Treatments on *Spartina* Performance

Similar to the initial measurements taken in greenhouse experiment 1, initial heights in this experiment were measured 3 weeks after planting (once treatment concentrations were established). Again, *S. cynosuroides* shoots were significantly taller than *S. alterniflora* shoots at this time. However, other plant characteristics (i.e. tiller diameter, number of leaves, and leaf area) did not differ between the two species. Both *S. alterniflora* and *S. cynosuroides* initial heights were greater in the second greenhouse experiment as compared to their measures in the first experiment ($p<0.0001$ and $p<0.0007$, respectively).

Survival

Spartina alterniflora survived better than in the first greenhouse experiment, but it still had significantly reduced survival in high salinity treatments with up to 75 % mortality (Figure 3.9, Tables 3.9 and 3.10). In contrast, *S. cynosuroides* target plants exhibited better survival with no significant differences among treatment combinations (Table 3.10). *Spartina cynosuroides* had 100% survival in five of six low salinity treatments and three of six high salinity treatments. While some transplants were replanted before treatment application (7 *S. alterniflora* and 20 *S. cynosuroides* were replanted), little mortality was observed after the first month of treatments (S. White, pers obs.). It is possible that the better survival of both *S. alterniflora* and *S. cynosuroides* in the second greenhouse experiment was due to their taller stature.

Plant growth parameters

Spartina alterniflora performance again mirrored its survival, with significantly reduced height, leaf area, number of plants, number of leaves, tiller diameter and biomass in all high salinity treatments (Tables 3.11 and 3.12, Figures 3.10 and 3.11). In contrast to the first experiment where sulfate treatments significantly affected *S. alterniflora* biomass measures, there were no sulfate treatment effects in this experiment and only one instance where there was an interaction between sodium and neighbor treatment (number of leaves). *Spartina cynosuroides* also showed decreased growth with increasing salinity, but fewer metrics were significantly affected (tiller diameter, number of plants, number of leaves, and biomass measures; Tables 3.13 and 3.14, Figures 3.12 and 3.13). However, *S. cynosuroides* belowground and total biomass was significantly reduced at high sulfate levels (Table 3.12). Neighbor presence or identity did not have strong impacts on growth, as *S. cynosuroides* height was the only parameter affected by neighbor treatment for either species.

Plant tissue % S and N:S ratios were affected by both sodium and sulfate treatments. For both species, % S values decreased whereas N:S ratios increased with increasing salinity (Tables 3.11 and 3.12, Figures 3.14 and 3.15). In addition, *S. cynosuroides* % N and C:N ratios decreased with increasing sodium levels and % C decreased with increasing sulfate levels. *Spartina cynosuroides* C:N ratio showed the only sodium x neighbor interaction, with a significant decrease when neighbors were absent.

Spartina alterniflora and S. cynosuroides elemental composition

Spartina plant tissues from the greenhouse experiments were analyzed for specific elemental concentrations in order to determine if there were any indicators of plant nutrients at toxic or deficient levels. We were particularly interested in the composition of *S. alterniflora* in the high salinity treatments as the high mortality observed in both of the greenhouse experiments was unexpected. The samples that were selected from the first greenhouse experiment were plants grown at 5 psu: 4 mM SO_4^{-2} conditions, which represent field brackish marsh conditions. Plant samples from the second greenhouse experiment were taken from treatments where target plants were without neighbors in the following NaCl:SO_4^{-2} conditions; 0 psu: 0 mM SO_4^{-2}, 0 psu: 28 mM SO_4^{-2}, 35 psu: 0 mM SO_4^{-2}, and 35 psu: 28 mM SO_4^{-2}. These observations were also compared to values for plants growing in the field (Chapter 2). We found that plants from the greenhouse experiments generally had lower concentrations of Al, Fe, Mg, Si, and Sr, and higher concentrations of K, Mn, and P than plants growing in the field. Calcium and Na concentrations were variable across both greenhouse and field experiments (Table 3.15).

Both species behaved similarly with regard to nutrient concentration changes among treatments in both greenhouse experiments. Ca, K, Mg, Mn and P were all greatest (for both *S. alterniflora* and *S. cynosuroides*) at low sodium: sulfate levels and reduced with increasing sodium: sulfate levels. Na was the only exception to this pattern with the greatest concentration for both species found at the highest sodium:sulfate level (35 psu).

DISCUSSION

The motivation behind the experiments presented here was to investigate the impacts of manipulating the abiotic and biotic environment of *S. alterniflora* and *S. cynosuroides* and then to extrapolate these results to help better explain field distributions along an estuarine salinity gradient. We were specifically interested in identifying the factors that limit *S. alterniflora* distribution in the upper estuarine reaches of the Altamaha River, as reciprocal transplant experiments in the field (Chapter 2) did not fully support either a competitive exclusion theory by *S. cynosuroides* in the brackish marsh or a strong sulfate requirement theory (i.e. insufficient resources in brackish environments). In the greenhouse experiments presented here, *S. cynosuroides* survival and growth was best at low salinities with no evidence for either sulfate or neighbor effects. These observations are consistent with field results and suggest that salinity tolerance sets the lower estuarine distribution for this species. However, we cannot rule out the possibility that whatever was negatively impacting *S. alterniflora* in the high salinity and sulfate treatments was also negatively impacting *S. cynosuroides* in these treatments (although these effects were not reflected in this species' survival data).

The unusual mortality and poor performance of *S. alterniflora* at salinities well within its physiological tolerance range is the most striking result of both the experiments described here. *Spartina alterniflora* survival was poorest in the high salinity treatments, which is the opposite of results from field experiments where *S. alterniflora* did best in higher salinity environments (Chapter 2). There was little evidence for either a sulfate requirement or neighbor interactions, although the poor survival of *S. alterniflora* in the greenhouse makes it difficult to draw definitive conclusions from these observations.

While it is possible that the shorter *S. alterniflora* plants were more susceptible to salt stress than *S. cynosuroides* in both experiments, the fact that other plant parameters (i.e. tiller diameter, number of leaves, and leaf area) were similar suggests that differences in height were probably not a main causative agent for poor growth and survival in *S. alterniflora*. Below we explore several possible factors that may have influenced *S. alterniflora* growth and success in the greenhouse: 1) we examine nutrient concentrations to determine whether the experimental nutrient conditions were maintained appropriately over the course of the experiment, or whether they were present in unusually high or low enough concentrations to inhibit growth, 2) we examine the macro- and micronutrient status of the *Spartina* plants to see whether they can shed light on not only the sulfate needs of *S. alterniflora* but also whether there were other nutrient requirements that were not being met in the greenhouse, and 3) we examine the influences that neighbors had to determine whether this can explain our results.

Experimental Nutrient Conditions & Plant Responses

Salinity and sulfate concentrations in both experiments reflected the treatments administered to the plants, although the salinities in the 35 psu treatments were typically below the target levels (Tables 3.2 and 3.8). All salinities were < 15 psu, which is well within the range for *S. alterniflora* (Phleger 1971, Haines and Dunn 1976, Parrondo et al. 1978, Bradley and Morris 1991) and should not have restricted growth. Bradley and Morris (1991) showed decreased height, leaf area and biomass measures as salinity treatments in their experiment were increased from 10 psu to 40 psu. Generally, *S. alterniflora*'s optimum growth is at salinities between 0-20 psu (Phleger 1971, Haines

and Dunn 1976, Parrondo et al. 1978, Bradley and Morris 1991) with reduced survival associated with salinities greater than 40-50 psu (Haines and Dunn 1976).

There was no evidence of reduced survival or growth at low sulfate conditions (in conjunction with low salinities), suggesting that sulfate availability was not restricting *S. alterniflora*'s growth. In fact, *S. alterniflora* performed poorly in treatments with greater sulfate concentrations.

Initial ammonium concentrations across the treatments in the first greenhouse experiment (~5 – 50 μM NH_4) were consistent with ammonium values reported from field experiments across a range of brackish and saline marshes (Table 3.2). Ewing et al. (1997) reported ammonium values between 16 and 18 μM in a Louisiana brackish marsh whereas Howard and Mendelssohn (2000) and Stribling and Cornwell (2001) measured values similar to those reported for Louisiana as well as considerably higher values in other Louisiana and Maryland brackish marshes (~263 μM and >600 μM respectively). Langis et al. (1991) reported ammonium concentrations between 15 and 78 μM in a California salt marsh and Stribling and Cornwell (2001) observed values ~ 47 μM in Maryland marshes with salinities greater than 15 psu. Final ammonium values in both greenhouse experiments were within these field ranges in treatments with low sodium and low or medium sulfate additions as well as in treatments of 5 psu : 4 mM SO_4^{-2} (greenhouse 1) and 0 psu: 28 mM (greenhouse 2).

At higher sodium and sulfate concentrations, where *S. alterniflora* had poor survival, final ammonium concentrations in both experiments increased well beyond any reported field values. These high ammonium values may be an artifact due to the greenhouse environment or possibly the result of desorption due to increasing sediment

salinities. Smart and Barko (1980) found similarly high NH_4-N values in a greenhouse sediment culture experiment with *S. alterniflora* using different field sediments as plant substrate. They reported ammonium concentrations of ~4300 µM in sediments originating from freshwater sites (~ 3 psu), 1030-4470 µM in brackish marsh sediments (~9-11 psu) and concentrations between 630 and 1310 µM in more marine sediments (> 26 psu). Both *Distichlis* and *S. alterniflora* aboveground biomass increased with increasing interstitial water NH_4-N concentration in their experiment. These higher than usual concentrations were explained by either the eutrophic nature of the sediment sources in the field and/or the lack of rooted plants in the substrate sampled from the field (Smart and Barko 1980). High ammonium concentrations are potentially toxic for plant growth, particularly under reduced soil conditions, as additional nutrients such as Fe and Mn become more soluble and thus detrimental for plant growth (Lambers et al. 1998). However, it is unlikely that the greenhouse soil conditions were reducing as sulfide concentrations were well below field ranges. These low sulfide concentrations suggest that the soils were fully oxidized and that potentially toxic nutrients were precipitated out of solution and thus less capable of being toxic to plants (Taiz and Zeiger 1998). Generally, toxic effects are avoided by plants' rapidly storing excess ammonium in their vacuoles, thus reducing the likelihood of the negative impacts brought about by ammonium accumulation (i.e. inhibition of photosynthesis, respiration, and shifts in plant carbohydrate status; (Taiz and Zeiger 1998, Britto et al. 2001, Kronzucker et al. 2001). There is also the possibility that the pH of the soils changed in this experiment as the plants used the available ammonium and extruded protons from their roots into the sediment (this is necessary in order for the roots to remain electrically neutral and thus

functional; Lambers et al. 1998). If this were the case, it would result in a reduction in soil pH and the potential mobilization of toxic ions (i.e. Al and Zn) as well as a reduction in the availability of required nutrients (P, Ca, Mg, K and Mo; Lambers et al. 1998).

Porewater ammonium concentrations were the same for both species in both greenhouse experiments. Thus, both plants should have exhibited the same growth inhibition as they experienced similar environmental conditions. However, since *S. alterniflora* survival at high salinities (where ammonium concentrations were the highest) was hindered to a greater extent than that of *S. cynosuroides* it may be reasonable to assume that *S. alterniflora* was more susceptible to these environmental condition than *S. cynosuroides*. It should be noted that although *S. cynosuroides* survival was better in these high ammonium conditions, *S. alterniflora* and *S. cynosuroides* growth parameters were similarly negatively affected. Further work needs to be conducted to find out at what level ammonium becomes toxic for *Spartina* species.

Plant Tissue Elemental Composition

Tissue C, N, and S concentrations were examined to explore the possibility that *Spartina* growth in the greenhouse was affected by unusual nutrient availabilities (i.e. limiting nutrients or toxic levels of nutrients), as this may have affected plant performance. Mendelssohn (1979) found that nitrogen fertilization in high salinity environments increased growth of *S. alterniflora* and suggested that the addition of nitrogen overcame the salt stress. Shea et al. (1975) found that growth limitation due to either high temperature or high salinity was counteracted when *S. alterniflora* was grown with high nitrogen fertilization. Tissue nitrogen concentrations that are adequate for *S. alterniflora* growth ranges between 0.66 and 0.80 % (Smart and Barko 1980). One

reported value for *S. cynosuroides* tissue is 0.30 % N (Beale and Long 1997) while Hopkinson and Schubauer (1984) reported a leaf value of 0.66 % N. *Spartina* N content from both greenhouse experiments surpass this range: *S. alterniflora* ranged from 1.69-3.10 % N and *S. cynosuroides* ranged from 1.42-2.17 % N. These observations suggest that nitrogen was not a limiting factor for either species.

Increasing sulfate concentrations significantly increased % S of both plants in both greenhouse experiments. *Spartina alterniflora* % S content can be as high as 1.2 % (Carlson and Forrest 1982) and as low as 0.13 % (Table 3.16). The sulfur content of *S. alterniflora* in both greenhouse experiments fall within this range (0.13-0.66 % S). The % S content of *S. cynosuroides* has not been reported previously but in this study it was similar to that of *S. alterniflora* (0.12-0.69 % S in both experiments). The sulfur content of both *Spartina* species were similar to those we measured in reciprocal transplant experiments in the field (Chapter 2). It is quite possible that reported *Spartina* tissue sulfur concentrations are underestimates, as it is likely that tissue sulfur is lost as dimethylsulfoniopropionate (DMSP) through sample preparation for nutrient analysis (R. Kiene, personal communication).

In the field, % S of both species was higher in the salt marsh environment, where both salinity and interstitial sulfate values were elevated compared to the brackish marsh (Chapter 2). In the greenhouse experiments the sulfur content of both species increased with increasing sulfate concentration but decreased with increasing sodium concentrations. If sulfate were limiting *S. alterniflora* growth we would have expected to see reduced performance of this species in low sulfate treatments. This is the opposite of what we found, as 1) *S. alterniflora* survival decreased as sulfate concentrations

increased, 2) of the plants that did survive in the high sulfate treatments, growth parameters (i.e. biomass) were decreased as compared to growth at lower sulfate concentrations, and 3) there was no significant sulfate effect on growth of *S. alterniflora* in the second greenhouse experiment.

Tissue N:S ratios are another useful way to evaluate a potential sulfate requirement. With increasing sodium concentrations, greenhouse N:S ratios of both species generally increased whereas field N:S ratios decreased. (The exception was during the first greenhouse experiment, where *S. alterniflora* N:S ratios decreased with increasing sodium concentrations.) However, when sodium concentrations are disregarded N:S ratios were higher in low sulfate treatments where sulfate is not readily available. N:S ratios from our greenhouse experiments ranged from 10-40:1 for *S. alterniflora* and 6-31:1 for *S. cynosuroides*, with lower ratios generally occurring in higher sulfate treatments (Tables 3.6, 3.7, 3.12, and 3.14). Plants can have molar N:S values as high as 30:1 and still have adequate sulfur (Epstein 1972). However, *S. alterniflora* N:S ratios were less than 3:1 in a low salinity Maryland marsh (Stribling 1994, Stribling and Cornwell 2001) which led these researchers to suggest that *S. alterniflora* growth is potentially restricted in low salinity environments due to sulfate nutrient limitations. Although both our field and greenhouse observations show increased N:S in *S. alterniflora* grown in low sulfate environments, both growth and survival were reduced in higher sulfate treatments, which is opposite of what would be expected if this plant had a sulfate requirement. In addition, *S. alterniflora*'s poor performance in the low sulfate and high sodium treatments (35 psu: 0 mM) is not likely a result of low sulfate availability as performance was equally poor in treatments with

either low or high sulfate concentrations at high salinity. Therefore, it appears that sulfate was not a limiting nutrient for *S. alterniflora* growth in these experiments.

Nutrient deficiencies can impact plant growth in a number of ways, such as 1) stunted growth (N, Mg, Ca, Mn), 2) disturbance of reproduction (P), 3) chlorosis of leaves due to disturbance of chlorophyll synthesis (Mg, Fe), 4) disturbance of water balance within the plant (K), and 5) necrosis of meristems and phloem (B; Taiz and Zeiger 1998). Plant tissue analysis often provides a more accurate indicator of nutrient deficiency than morphological symptoms found in plants (Taiz and Zeiger 1998). Bandyopadhyay et al. (1993) and Bradley and Morris (1991) found that excessive salinities and inundation can exacerbate nutrient deficiencies by inhibiting nutrient uptake in marsh plants.

We were particularly interested in plant tissue Fe, Mg, K, P, and Zn concentrations as these nutrients strongly influence plant growth and success. Some variation between field and greenhouse environments is to be expected, as the growing conditions are not the same. In fact, we might expect plants to do better in the greenhouse as it is a more controlled environment. In comparing our greenhouse nutrient results to our field values (and to previously published field values) we found that Al, Fe, Mg, and Si concentrations were higher in field samples of both species whereas Ca (in *S. cynosuroides*), and K, Mn, Na, Zn, and P concentrations of both species were higher in the greenhouse experiments (Table 3.15). It may be that nutrient deficiencies (Fe) and/or toxicities (Zn) were accentuated in high salinity treatments in the greenhouse, leading to decreased plant performance; below we explore all the nutrients in greater detail. It should be noted that the following discussion is based on nutrient comparisons between

the *Spartina* species and published literature values for this species (primarily *S. alterniflora*) as well as published crop nutrient values (typically rice plants, which are also C4 grasses). Although we recognize that there may be significant variability between *Spartina* and rice plant nutrient requirements, these comparisons offer a general framework in which to assess *Spartina* nutrient status.

It appears that both aluminum and calcium were in sufficient supply for successful *Spartina* growth in the greenhouse when compared to literature values. Aluminum concentrations in both experiments (~18-200 ppm) were considerably below the ranges reported from our field work (~1542-3047 ppm; Table 3.15) as well as those reported in the literature (~800-4000 ppm; Table 3.15) but within the range of values reported by Taiz and Zeiger (1998) for successful plant growth. It is not know whether these species have an unusually high Al requirement. Generally Al is not considered to be an essential element and it is unlikely that low levels of aluminum would cause reduced survival and growth. Field and greenhouse Ca concentrations were comparable and within reported field ranges (~1500-3200 ppm), suggesting that Ca was also not limiting.

Tissue nutrient concentrations of Fe and Mg for both *Spartina* species in both greenhouse experiments were well below both our field observations and the ranges reported in the literature. Iron concentrations did not show statistically significant patterns with increasing salinity whereas Mg values for both *Spartina* species showed a trend of decreasing concentrations with increasing salinity in the greenhouse. It is possible that greenhouse Fe and Mg availability may have been deficient (as compared to

field ranges) and the limited availability combined with the harsher conditions found in the higher salinity treatments may have inhibited *Spartina* plant performance.

Potassium, Mn, Na, and P nutrient requirements all appeared to be satisfied as our reported values were either above or within our field and reported literature ranges. Potassium values for *S. alterniflora* plants grown in higher salinity treatments were within field and literature ranges, whereas those grown in moderate or low salinity and sulfate treatments were considerably greater than either field or reported values (1000-14,076 ppm). However, since this species performed well in these treatments, K is apparently not toxic at these high concentrations. *Spartina cynosuroides* values were lower than *S. alterniflora* values in all treatments and were within the reported range. Manganese concentrations in the greenhouse were comparable to our field observations, but considerably greater than previously reported values (~25-80 ppm). *Spartina alterniflora* had the highest Mn concentrations in low salinity treatments in the greenhouse, which argues against high Mn as the cause of reduced *S. alterniflora* performance in high salinities. Our results show increasing Na concentrations with increasingly salinity in the greenhouse (~932-16, 496 ppm) and this is similar to our field results. At no point, however, do these values seem excessively high in comparison to literature values that range from 300-117,708 ppm. *Spartina* plants grown in the greenhouse had generally higher P values (~1930-6829 ppm) than both our field (~666-2335 ppm) and the reported literature ranges (~400-2000 ppm), and it is possible that high P concentrations can negatively affect plant growth. Yeo and Flowers (Yeo and Flowers 1980) found that halophyte growth could be more inhibited by high concentrations of P, rather than Na, as excess P can contribute to additional micronutrient

deficiencies (i.e. K deficiency). However, this is not likely to be the case in our experiments as K was not limiting for either species and survival was greatest where P concentrations were largest.

Spartina alterniflora, as a halophytic plant, is able to accumulate fairly high concentrations of salts in its tissues for osmotic adjustment through compartmentalization of ions in vacuoles and the production of compatible solutes in the cytoplasm (Flowers 1985). The high concentrations we observed for ions such as K, Mn, and P in *Spartina* plants grown under manipulated salinity and sulfate conditions in the greenhouse could potentially have inhibited nutrient uptake by competitively inhibiting other necessary nutrients (i.e. N or S) from binding to root membrane transporters. This is unlikely to be the case as our reported N and S tissue nutrient concentrations were within the range of critical nutrient levels reported for this species. When adequate nitrogen is available there is the possibility of a secondarily limiting nutrient to restrict growth (Liebig 1847, Valiela 1995). However, P, K, and S tissue values were all either greater than reported ranges or within the general range of critical nutrient availability. Iron or Mg limitation is possible, however, as these values are below previously reported ranges. Further work on *Spartina* growth under manipulated Fe and Mg concentrations is needed to address whether these elements could limit the growth of these plants.

It is possible that high Zn concentrations were the cause of the poor survival observed in this study. *Spartina* Zn tissue concentrations in both greenhouse experiments (~26-105 ppm) were much greater than previously reported values (~8-20 ppm). (Zinc concentrations from our reciprocal field transplants were not detectable.) Although Zn concentrations in both species were high, concentrations in *S. alterniflora* were greater

than *S. cynosuroides* for all but one treatment combination (except 5 psu: 4 mM SO_4^{-2}). One inconsistency is that Zn concentrations were highest in plants grown at low salinity: low sulfate levels but it is possible that with the added stress of high salts Zn could have negatively affected *S. alterniflora* success in higher salinity environments. The concentrations of Zn found in *S. cynosuroides* were lower and so may not have affected survival (although growth measures were still reduced).

While we did not test soil nutrient concentrations, it is likely the soils in which these plants grew contained high levels of Zn. Zinc is considered a heavy metal and when concentrations exceed trace levels in the soil this element can be extremely toxic (Taiz and Zeiger 1998). Heavy metal toxicity (including essential micronutrients such as Zn, Cu, Co, and Ni as well as non-nutrient metals Hg, Pb, Ca, Ag, Cr) results from their ability to cause oxidative damage to tissue (Stohs and Bagchi 1995). Some plants do have the ability to adapt to metal-rich soils (Antonovics and Bradshaw 1970) and other plants have the ability to accumulate high concentrations of metals (called hyperaccumulators) in their vacuoles without undue tissue damage (Baker and Brooks 1989). Little work has been completed on whether *Spartina* plants are genetically adapted to high metal conditions, but Qu et al. (2003) observed that *S. patens* seems able to tolerate high Pb concentrations. Currently, it is unclear whether *S. alterniflora* or *S. cynosuroides* can be considered hyperaccumulators and handle possible high trace metal concentrations in their tissues without physiological damage. Additional research would be useful to better describe either species' ability to tolerate trace metals. This information might then help explain whether Zn toxicity was a problem for *Spartina* growth in the greenhouse.

The ratio of cations in plant tissue to tissue Na concentrations is important because of interactions with nutrient uptake processes and plant functionality (Adam 1990, Donovan et al. 1997). The ability to maintain K uptake in an environment with high Na concentrations is a defining characteristic of salinity tolerance (Adam 1990). Sodium and other cations (i.e. K, P) in the soil matrix often compete for available binding sites on root membranes (Phleger 1971, Niu et al. 1995) and thus K:Na and/or Cl:Na ratios can describe environmental constraints for plant growth. K:Na leaf values that are greater than 1 are typically found in glycophtic plants (low salinity tolerance) whereas K:Na values that are less than 1 are more typical of halophytic plants (high salinity tolerance, ie. *Sarcobatus*) (Gorham et al. 1980, Glenn and Oleary 1984, Donovan et al. 1997). The K:Na molar ratios for *S. alterniflora* can range from ~0.7 to 0.9 in either the brackish marsh or salt marsh environment (Smart and Barko 1980). The K:Na molar ratios for *S. alterniflora* plants in our greenhouse environment were quite variable and ranged from 0.4-2.9 whereas in the field they were 0.9 and 1.5 in the salt and brackish marsh environments, respectively. As *S. alterniflora* is a halophyte, we expect to find K:Na ratios that are lower than 1, however, this was not always the case in our studies. These higher values may be a result of *S. alterniflora*'s ability to exert strong control over ion accumulation via processes of selective exclusion and secretion of Na^+ over K^+ (Smart and Barko 1980, Bradley and Morris 1991). *Spartina alterniflora* Na^+ concentrations generally increased with increasing salinity while K^+ concentrations decreased, resulting in decreasing K:Na ratios. *Spartina alterniflora* may be increasing plant tissue concentrations of Na^+ over K^+ in order to synthesize compatible solutes that aid in salt-tolerance. *Spartina cynosuroides* K:Na ratios ranged from 0.4-1.9 in the

greenhouse, whereas in the field they were lower than those observed in *S. alterniflora* (0.82 and 0.67 in the salt and brackish environments, respectively). While there are no previous observations of K:Na in *S. cynosuroides*, this species is considered to be less salt tolerant than *S. alterniflora* and thus more similar to glycophytic than halophytic plants. It is therefore surprising for *S. cynosuroides* ratios to be less than those of *S. alterniflora*.

While it appears that macronutrients (nitrogen and sulfur) were not limiting *Spartina* growth under these manipulated greenhouse conditions, further work needs to be completed in order to better understand how other elements influence *Spartina* growth. Aluminum, iron and magnesium concentrations were all lower in the greenhouse environment than in the field, whereas phosphorous and zinc concentrations were considerably greater than field observations. It is quite possible that iron limitation or zinc toxicity negatively influenced *S. alterniflora* growth in high salinity treatments in this study.

Further efforts exploring *Spartina* micro- and macronutrient limitations, genetic variability and osmolyte responses could be useful in more clearly defining mechanisms controlling plant distributions. It is important to find out what intraspecific variability is present among sampled *Spartina* populations as it is conceivable that different genotypes of *S. alterniflora* and *S. cynosuroides* can have variable physiological tolerances to salinity/sulfate concentrations. Hester et al. (1996, 1998, 2001) found significant intraspecies variability in salinity tolerance of *S. alterniflora* and *S. patens*. They also found that morphological differences between *S. alterniflora* genotypes were valuable in assessing salt tolerance. This type of genetic variability needs to be better understood in order to make sure that any greenhouse experiment utilizing marsh plants does not

confound the results by using either sample plants with multiple tolerance levels or plants with unusually low/high tolerances. In addition to understanding *Spartina* genetic variability, it would be interesting to understand how different osmolytes vary with salinity and sulfate treatments. The roles of dimethylsulphoniopropionate (DMSP), glycine betaine and proline as osmolytes in *Spartina* have been previously investigated (Cavalieri and Huang 1981, Cavalieri 1983, Dacey et al. 1987, Otte and Morris 1994, Colmer et al. 1996, Mulholland and Otte 2000). While the concentration of DMSP in the cystol of *S. alterniflora* plants is estimated to be 20-70 mM (Dacey et al. 1987) it would be interesting to determine if DMSP concentrations vary within *Spartina* species across salinity and sulfate gradients and discover if variations in DMSP concentrations might influence *Spartina* distribution and performance under changing salinity or sulfate regimes.

Neighbor Impacts on Spartina Plant Parameters

Neighbor presence and identity was not a defining parameter for *Spartina* survival and growth in this study. In the experiments described here, inter-and intraspecific competitive interactions were rarely significant. *Spartina alterniflora* tiller diameter measures showed an interaction effect between sodium and neighbors (Table 3.11) whereas *S. cynosuroides* height was negatively impacted by neighbor presence in treatments with low salinities and low sulfate (Table 3.12). The lack of multiple instances of neighbor influences (competition or facilitation) on *Spartina* growth under any combination of sodium and sulfate concentration suggests that abiotic factors controlled performance rather than biotic interactions. This fits our field observations of *S. cynosuroides*, which were unaffected by neighbor presence. However, neighbor

presence did result in a reduction in *S. alterniflora* performance in the salt marsh, which was not observed in the greenhouse. In general, however, the lack of a response to neighbor manipulation in our greenhouse experiments is generally consistent with results from the field and suggests that neighbors are not strong mechanisms defining the upper or lower distributions of either *S. cynosuroides* or *S. alterniflora* along the salinity gradient of the Altamaha River.

CONCLUSION

The purpose of our greenhouse manipulations was to better define the response of *Spartina* to changes in sodium, sulfate and neighbor presence, and then integrate these findings with previous results from reciprocal transplant studies in the field. Our goal was to develop a general model of the mechanisms that control *Spartina* distributions along an estuarine salinity gradient. The results from these experiments indicate that increasing salinity had a negative impact on the survival and growth of both *S. alterniflora* and *S. cynosuroides* in the greenhouse environment, whereas plant-plant interactions and sulfate concentrations were less important. The improved survival and performance of *S. cynosuroides* in both the low salinity treatments in the greenhouse and in the brackish marsh support the assertion that abiotic conditions, most likely high salinities, are the dominant mechanism limiting this species' lower estuarine distribution.

We had hoped greenhouse experiments would enable us to get a better understanding of how *S. alterniflora* grows in low salinity and low sulfate conditions, as the success of transplants of this species into brackish marsh conditions in the field was poor. *Spartina alterniflora*'s survival and growth in low salinity greenhouse conditions was good, although neither neighbor nor sulfate interactions were observed to help

explain the poor survival in the field. The greater mortality of this species in high salinity treatments was unanticipated, and mechanisms such as plant-plant interactions and/or sulfate limitations did not explain this result. We cannot eliminate the possibility that this mortality may be a result of inhibitive soil nutrient conditions (either too high or too low) and further research exploring this nutrient angle is warranted. Given these greenhouse results, we were able to support the field conclusion that *S. cynosuroides*' lower distribution is controlled by abiotic conditions; however, the mechanisms influencing the upper distribution of *S. alterniflora* are still unclear.

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Table 3.1. Nutrient fertilizer concentrations in ppm and g-weight added weekly (500ml) to each pot.

Element	**N**	**Mg**	**Na**	**S**	**B**	**Cu**	**Fe**	**Mn**	**Mo**	**Zn**
Conc. (ppm)	1.95	2.25	4.2	2.97	0.3	0.15	1.5	0.84	0.15	0.24
Amt. added/pot (mg)	0.975	1.13	2.10	1.48	0.15	0.075	0.75	0.42	0.075	0.12

Table 3.2. Interstitial water nutrient concentrations at each treatment level (mean values ± S.E.) in greenhouse experiment #1. Target species identity does not influence interstitial nutrients, thus data represents concentrations at the specific treatment regardless of species identity. Treatments are NaCl (psu): SO_4^{-2} (mM). Different letters indicate significant differences among treatments. Significant results ($p < 0.05$) are indicated in boldface. nd=undetectable.

NUTRIENT **Mean ± S.E; N**	**1** **Trt: 0,0**	**2** **Trt: 0,4**	**3** **Trt: 5,0**	**4** **Trt: 5,4**	**5** **Trt: 5,28**	**6** **Trt: 35,0**	**7** **Trt: 35,4**	**8** **Trt: 35,28**	**p-value**
NH4 (uM); N									
Initial	4.8 ± 0.5 [a]; 5	9.9 ± 4.1 [a]; 6	16.2 ±7.4 [a]; 6	10.8 ± 3.4 [a]; 6	51.7 ± 29.6 [a]; 6	35.3 ± 8.3 [a]; 6	10.9 ± 2.4 [a]; 6	7.6 ± 1.5 [a]; 6	0.0807
Final	19.8 ± 4.9 [a]; 8	23.5 ± 11.6 [a]; 4	71.7 ± 9.0 [a]; 4	1257 ± 190 [b]; 4	877 ± 79 [b]; 5	3424 ± 214 [c]; 4	4941 ± 461 [d]; 3	3877 ± 367 [c]; 3	**<0.0001**
$SO4^{-2}$ (mM)	0.47 ± 0.28 [a]; 4	2.9 ± 0.4 [ab]; 2	0.70 ± 0.21 [a] ; 4	5.2 ± 2.5 [ab]; 4	13.7 ± 2.1 [c]; 4	1.1 ± 0.1 [a]; 2	3.2 ± 0 [ab]; 2	12.7 ± 1.9 [bc]; 2	**<0.0001**
H_2S (uM)	5.4 ± 3.1 [a]; 4	3.4 ± 1.3 [a]; 4	5.8 ± 0.9 [a]; 2	3.8 ± 0.5 [a]; 2	1.4 ± 0.9 [a]; 2	nd	nd	nd	0.7479
Salinity	0 ± 0 [a]; 12	0.08 ± 0.08 [a]; 12	3.5 ± 0.5 [b]; 12	4.3 ± 0.3 [b]; 12	4.7 ± 0.4 [b]; 12	20.4 ± 1.5 [c] ;10	23.4 ± 1.0 [c]; 9	23.8 ± 1.3 [c]; 9	**<0.0001**

Table 3.3. Final ionic strengths (means ± SE) of both greenhouse experiments by treatment. Treatments are NaCl (psu): SO_4^{-2} (mM). Greenhouse experiment 1 did not have a 0 psu: 28 mM SO_4^{-2} and greenhouse experiment 2 only had four treatments.

	Treatment								
	0,0	**0,4**	**0,28**	**5,0**	**5,4**	**5,28**	**35,0**	**35,4**	**35,28**
Greenhouse Experiment 1	9.4×10^{-4} $\pm 1.4 \times 10^{-4}$	5.8×10^{-3} $\pm 2.1 \times 10^{-4}$	na	4.9×10^{-3} $\pm 3.7 \times 10^{-4}$	1.4×10^{-2} $\pm 1.3 \times 10^{-4}$	3.2×10^{-2} $\pm 1.1 \times 10^{-3}$	2.3×10^{-3} $\pm 1.1 \times 10^{-3}$	2.9×10^{-2} $\pm 7.1 \times 10^{-4}$	4.9×10^{-2} $\pm 1.3 \times 10^{-3}$
Greenhouse Experiment 2	2.7×10^{-4} $\pm 1.3 \times 10^{-5}$	na	1.7×10^{-2} $\pm 6.8 \times 10^{-4}$	na	na	na	1.4×10^{-2} $\pm 7.4 \times 10^{-4}$	na	3.4×10^{-2} $\pm 9.6 \times 10^{-4}$

Table 3.4. ANOVA table for *S. alterniflora* plant parameters from greenhouse experiment #1. Significant results ($p < 0.05$) are indicated in boldface.

Growth Characteristic	**Sodium**	**Sulfate**	**Sodium X Sulfate**
Height	**<0.0001**	0.1996	0.1756
Tiller	**0.0014**	0.9636	0.7251
No. Leaves	**<0.0001**	0.3889	0.1660
Photosynthetic Area	**0.0003**	0.3948	0.2385
Aboveground Biomass	**<0.0001**	**0.0017**	0.3809
Belowground Biomass	**0.0013**	**0.0178**	0.5809
Total Biomass	**<0.0001**	**0.0029**	0.4088
Above:Belowground Biomass	**<0.0001**	**0.0172**	0.5988
Number of plants	**0.0361**	0.2838	0.5796
% N	0.9138	0.1568	0.9235
% C	**0.0008**	**0.0252**	0.0876
% S	0.1586	**0.0102**	0.3664
C:N Ratio	0.9819	0.0916	0.6890
N:S Ratio	0.1139	0.0766	0.5723

Table 3.5. ANOVA table for *S. cynosuroides* plant parameters from greenhouse experiment #1. Significant results ($p < 0.05$) are indicated in boldface.

Growth Characteristic	**Sodium**	**Sulfate**	**Sodium X Sulfate**
Height	**<0.0001**	**0.0463**	0.0868
Tiller	**<0.0001**	0.6261	0.9053
No. Leaves	**<0.0001**	0.3428	0.4320
Photosynthetic Area	**<0.0001**	**0.0128**	0.1662
Aboveground Biomass	**<0.0001**	0.2443	0.5893
Belowground Biomass	**<0.0001**	0.1985	0.6955
Total Biomass	**<0.0001**	0.1272	0.5258
Above:Belowground Biomass	**<0.0001**	0.5266	0.2605
Number of plants	**<0.0001**	0.8541	0.3022
% N	0.3225	0.4401	0.6688
% C	0.0903	0.5883	0.4531
% S	**0.0013**	0.1043	0.1594
C:N Ratio	0.4209	0.4976	0.6282
N:S Ratio	**0.0049**	0.8176	0.4041

Table 3.6. Plant metrics for greenhouse experiment #1: *S. alterniflora* growth across treatments (mean values ± S.E.; N). 100 % of *S. alterniflora* plants died in treatments 7 & 8. Treatments are NaCl (psu): SO_4^{-2} (mM). Different letters indicate significant differences among treatments. ns= no sample.

METRIC **Mean ± S.E.; N**	**(1)** **Trt:0,0**	**(2)** **Trt: 0,4**	**(3)** **Trt: 5,0**	**(4)** **Trt: 5,4**	**(5)** **Trt: 5, 28**	**(6)** **Trt: 35, 0**
Height (m/pot)	15.3 ± 1.5[a]; 7	15.9 ± 0.9[a]; 7	15.9 ± 0.6[a]; 7	13.5 ± 1.8[a]; 7	10.19 ± 1.43[a]; 7	0.50[b]; 1
Tiller (mm/pot)	26.1 ± 2.5[a]; 7	26.0 ± 1.7[a]; 7	25.8 ± 2.8[a]; 7	24.1 ± 1.8[a]; 7	19.29 ± 1.46[a]; 7	5[b]; 1
No. Leaves/ pot	62.8 ± 2.3[a]; 7	61.4 ± 2.6[a]; 7	57 ± 5.0[a]; 7	53.4 ± 3.9[a]; 7	47.57 ± 4.98[a]; 7	10[b]; 1
Photo. Area (cm^2/pot)	7062 ± 1085[a]; 7	8123 ± 601[a]; 6	6182 ± 749[a]; 7	6445 ± 1310[a]; 7	3621 ± 491[a]; 7	396[b]; 1
Above Bio (g/pot)	74.4 ± 4.2[a]; 7	85.8 ± 5.4[a]; 7	74.5 ± 6.9[a]; 7	66.9 ± 10.7[a]; 7	32.8 ± 0.3[b]; 7	1.7[c]; 1
Below Bio (g/pot)	33.0 ± 2.7[a]; 7	28.3 ± 2.9[a]; 7	26.4 ± 2.8[ab]; 7	20.5 ± 4.3[ab]; 7	12.6 ± 2.2[bc]; 7	3.6[c]; 1
Total Bio (g/pot)	107 ± 6[a]; 7	114 ± 8[a]; 7	101 ± 9[a]; 7	87.5 ± 15.0[ab]; 7	45.5 ± 9.0[b]; 7	5.3[c]; 1
A:B ratio	2.3 ± 0.1[a]; 7	3.2 ± 0.3[a]; 7	2.9 ± 0.2[a]; 7	3.5 ± 0.2[a]; 7	2.5 ± 0.3[a]; 7	0.48[b]; 1
No. Plants/plot	19 ± 3.0[a];7	18.1 ± 2.6[a];7	18.8 ± 0.7[a]; 6	15.2 ± 2.8[a]; 7	13.1 ± 1.6[a]; 7	2[b]; 1
% Nitrogen	1.7[a]; 1	2.1 ± 0.1[a]; 3	1.7 ± 0.2[a]; 5	2.1 ± 0.1[a]; 2	2.1 ± 0.1[a]; 4	ns
% Carbon	46.2[a]; 1	45.7 ± 0.1[a]; 3	45.2 ± 0.2[ab]; 5	43.3 ± 0.4[c]; 2	44.3 ± 0.1[bc]; 4	ns
% Sulfur	0.20[ab]; 1	0.34 ± 0.07[ab]; 3	0.24 ± 0.04[a]; 5	0.50 ± 0.07[b]; 2	0.46 ± 0.02[b]; 4	ns
C:N	30.1[a]; 1	25.1 ± 1.5[a]; 3	31.6 ± 2.7[a]; 5	23.8 ± 1.5[a]; 2	23.9 ± 1.9[a]; 4	ns
N:S	20.3[a]; 1	16.1 ± 4.5[a]; 3	17.2 ± 0.8[a]; 5	9.9 ± 0.7[a]; 2	10.9 ± 0.4[a]; 4	ns

Table 3.7. Plant metrics for greenhouse Exp #1. *S. cynosuroides* differences between treatments (mean values ± S.E.; N). Treatments are NaCl (psu): SO_4^{-2} (mM). Different letters indicate significant differences among treatments.

METRIC Mean (±S.E.); N	(1) Trt:0,0	(2) Trt: 0,4	(3) Trt: 5,0	(4) Trt: 5,4	(5) Trt: 5, 28	(6) Trt: 35, 0	(7) Trt: 35, 4	(8) Trt: 35, 28
Height (m/pot)	19.5 ± 1.6 [a]; 7	17.4 ± 3.0 [a]; 7	16.0 ± 1.1 [a]; 7	17.9 ± 1.8 [a]; 7	11.8 ± 1.2[a]; 6	1.6 ± 0.3 [b]; 7	1.3 ± 0.1 [b]; 6	1.31 ± 0.25[b]; 7
Tiller (mm/pot)	22.1 ± 1.2 [a]; 7	21.0 ± 3.0 [a]; 7	23 ± 0.8 [a]; 7	26.2 ± 2.7 [a]; 7	22.3 ± 1.9 [a]; 6	12.1 ± 1.9 [b]; 7	9.5 ± 1.4 [b]; 6	8.8 ± 1.7 [b]; 7
No. Leaves/ pot	34.7 ± 1.4 [a]; 7	29.1 ± 4.5 [ab]; 7	35.5 ± 1.5 [a]; 7	39.7 ± 5.1 [a]; 7	37.0 ± 1.5 [a]; 6	21.4 ± 2.9 [bc]; 7	16.6 ± 2.5 [bc]; 6	15.5 ± 3.0 [c]; 7
Photo. Area (cm^2/pot)	4074 ± 387 [a]; 7	3065 ± 564 [a]; 7	4168 ± 365 [a]; 7	4406 ± 664 [a]; 7	3009 ± 326 [a]; 6	721.0 ± 130.6 [b]; 7	463 ± 60.9 [b]; 6	483 ± 80 [b]; 7
Above Bio (g/pot)	83.9 ± 9.4 [a]; 7	72.6 ± 13.2 [a]; 7	59.7 ± 5.2 [a]; 7	64.5± 8.2 [a]; 7	36.6 ± 3.5 [a]; 6	4.2 ± 0.9 [b]; 7	3.4 ± 0.4 [b]; 6	3.0 ± 0.5 [b]; 7
Below Bio (g/pot)	38.9 ± 6.1 [a]; 7	38.6 ± 7.7 [a]; 7	46.5 ± 5.6 [a]; 7	42.4 ± 5.9 [a]; 7	23.2 ± 5.4 [a]; 6	4.8 ± 0.8 [b]; 7	3.8 ± 0.8 [b]; 6	3.8 ± 0.8 [b]; 7
Total Bio (g/pot)	122 ± 14[a]; 7	111 ± 20 [a]; 7	106 ± 10[a]; 7	106.9 ± 13.0[a]; 7	54.6 ± 9.8 [a]; 7	9.0 ± 1.6 [b]; 7	6.8 ± 0.8 [b]; 7	6.8 ± 1.3 [b]; 7
A:B ratio	2.3 ± 0.2 [ab]; 7	2.1 ± 0.1 [ab]; 7	1.3 ± 0.1 [ab]; 7	1.5 ± 0.1 [a]; 7	1.7 ± 0.2 [ab]; 6	0.88 ± 0.11 [b]; 7	1.3 ± 0.4 [ab]; 6	0.84 ± 0.08 [b]; 7
No. Plants/plot	16.8 ± 1.4 [a]; 7	14.5 ± 2.3 [a]; 7	15.5 ± 1.7 [a]; 7	19.8 ± 2.3 [a]; 7	16.1 ± 3.1 [a]; 6	3 ± 0.58 [b]; 7	2.8 ± 0.4 [b]; 6	3.1 ± 0.7 [b]; 7
% Nitrogen	1.8 [a]; 1	2.1 ± 0.2 [a]; 3	1.7 ± 0.1 [a]; 3	1.7 ± 0.1 [a]; 4	1.7 ± 0.1 [a]; 4	1.5 [a]; 1	2.0 ± 0.5 [a]; 2	2.0 ± 0.1 [a]; 4
% Carbon	45.4 [a] ; 1	44.6 ± 0.2 [a]; 3	44.1 ± 0.2 [a]; 3	44.4 ± 0.1 [a]; 4	44.2 ± 0.3 [a]; 4	44.8 [a]; 1	44.3 ± 0.2 [a]; 2	44.6 ± 0.3 [a]; 4
% Sulfur	0.31 [ab]; 1	0.6 ± 0.1 [a]; 3	0.45 ± 0.01 [ab]; 3	0.5 ± 0.02 [ab]; 4	0.43 ± 0.11 [ab]; 4	0.15 [b]; 1	0.18 ± 0.03 [b]; 2	0.24 ± 0.03 [b]; 4
C:N	28.4 [a]; 1	24.8 ± 2.7 [a]; 3	29.4 ± 1.1 [a]; 3	31.6 ± 4.1 [a]; 4	29.7 ± 1.1 [a]; 4	33.0 [a];1	26.9 ± 6.7 [a]; 2	25.4 ± 0.8 [a]; 4
N:S	13.9 [a]; 1	7.3 ± 1.2 [a]; 3	8.8 ± 0.3 [a]; 3	7.4 ± 0.9 [a]; 4	13.7 ± 6.1 [a]; 4	24.4 [a]; 1	25.6 ± 2.3 [a]; 2	20.8 ± 2.8 [a]; 4

Table 3.8. Interstitial water nutrient concentration at each treatment level (mean values ±S.E.) in greenhouse experiment #2. Target species identity does not influence interstitial nutrients, thus data represents concentrations at the specific treatment regardless of species identity. Different letters indicate significant differences among treatments within either the initial or final means (ns=no sample). Significant results ($p < 0.05$) are indicated in boldface.

Treatment Nutrient (N)	(1) Trt:0,0 w/opp	(2) Trt: 0,0 w/same	(3) Trt: 0,0 Alone	(4) Trt: 0,28 w/opp	(5) Trt: 0,28 w/same	(6) Trt: 0,28 Alone	(7) Trt: 35, 0 w/opp	(8) Trt: 35, 0 w/same	(9) Trt: 35,0 Alone	(10) Trt: 35,28 w/opp	(11) Trt: 35,28 w/same	(12) Trt: 35,28 Alone	p-value
NH4 (uM) Initial (6)	342.3± 88.0 [ab]	250 ± 41 [a]	577 ± 89 [abc]	699 ± 144 [abc]	386 ± 161[ab]	1077 ± 199 [c]	383 ± 53[ab]	719 ± 139[abc]	807 ± 65[ab]	558 ± 79[abc]	729 ± 71[abc]	955 ± 77[c]	**<0.0001**
Final (6)	2.5 ± 0.5[a]	2.0 ± 0.3 [a]	4.1 ± 1.3 [a]; 3	0.81 ± 0.11 [a]	0.77 ± 0.09 [a]	19.8 ± 6.1 [a]; 5	340 ± 45[ab]; 7	684 ± 127[b]	669 ± 69 [b]	444 ± 50[b]; 7	643 ± 44[b]; 5	510 ± 195[b]; 7	**<0.0001**
$SO4^{-2}$ (mM) (4)	ns	ns	0.41 ± 0.05 [a]; 2	8.0 ± 1.9 [ab]	6.7 ± 0.9 [ab]	9.9 ± 1.5 [b]	ns	ns	ns	8.7 ± 1.8 [ab]	10.3 ± 1.5 [b]	8.6 ± 1.3 [ab]	**0.0367**
H_2S (uM) (2)	ns	ns	5.63 [a]; 1	1.4 ± 0.9 [a]	1.7 ± 1.3 [a]	1.64 [a]; 1	ns	ns	ns	0.42 [b]; 1	25.4 [b]; 1	16.7 ± 0.2 [b]	**0.0032**
Salinity (psu) (12)	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0.67 ± 0.26 [a]	0.92 ± 0.42 [a]	1.1 ± 0.4 [a]	14.0 ± 1.2 [b]; 13	12.6 ± 1.3 [b]; 13	16.5 ± 1.1 [b]; 14	13.9 ± 1.5 [b]; 13	16.5 ± 0.9 [b]	17.3 ± 0.8 [b]; 13	**<0.0001**

Table 3.9. Percent target plant survival by treatment in greenhouse experiment #2.

	Nutrient (NaCl, SO_4^{-2}) and neighbor (w/SC, w/SA, or alone) treatment											
Species	**(1) 0,0 w/SC**	**(2) 0,0 w/SA**	**(3) 0,0 Alone**	**(4) 0,28 w/SC**	**(5) 0,28 w/SA**	**(6) 0,28 Alone**	**(7) 35, 0 w/SC**	**(8) 35, 0 w/SA**	**(9) 35,0 Alone**	**(10) 35,28 w/SC**	**(11) 35,28 w/SA**	**(12) 35,28 Alone**
S. alterniflora	100%	100%	75%	50%	100%	75%	50%	50%	25%	75%	25%	50%
S. cynosuroides	100%	100%	100%	100%	100%	75%	100%	100%	75%	50%	75%	100%

Table 3.10. Logistic regression analysis of mortality by *S. alterniflora* and *S. cynosuroides* in greenhouse experiment # 2.

Main Effects	***S. alterniflora***		
	df	**Wald χ^2**	**P**
Sodium	1	6.82	**0.0090**
Sulfate	1	0.851	0.6535
Neighbor Id.	2	0.110	0.741
Main Effects	***S. cynosuroides***		
	df	**Wald χ^2**	**P**
Sodium	1	1.84	0.1750
Sulfate	1	0.476	0.7882
Neighbor Id.	2	1.84	0.1750

Table 3.11. ANOVA table for *S. alterniflora* plant parameters from greenhouse experiment #2. Significant results ($p < 0.05$) are indicated in boldface.

Growth Characteristic	**Sodium**	**Sulfate**	**Neighbor**	**Sodium x Neighbor**	**Sulfate x Neighbor**	**Sodium x Sulfate**	**Sodium x Sulfate x Neighbor**
Height	**<0.0001**	0.7488	0.2438	0.0081	0.9584	0.8353	0.1568
Tiller	**<0.0001**	0.4832	0.2132	**0.0231**	0.9569	0.3712	0.3792
No. Leaves	**<0.0001**	0.4821	0.8198	0.3586	0.9645	0.7137	0.5683
Photosynthetic Area	**<0.0001**	0.0572	0.8345	0.3855	0.6921	0.6343	0.2239
Aboveground Biomass	**<0.0001**	0.7055	0.4051	0.1648	0.5237	0.6745	0.2452
Belowground Biomass	**0.0001**	0.9588	0.5486	0.1130	0.9211	0.3751	0.1103
Total Biomass	**0.0002**	0.9684	0.7106	0.0828	0.7888	0.6084	0.0589
Above:Belowground Biomass	0.9401	0.8509	0.0564	0.7539	0.5233	0.5597	0.7938
Number of plants	**<0.0001**	0.4423	0.5462	0.0789	0.8097	0.8126	0.2419
% N	0.3039	0.4400	0.9806	0.0595	0.1802	0.7924	0.2366
% C	0.1610	0.1840	0.1752	0.2733	0.4487	0.2597	0.3161
% S	**<0.0001**	**0.0042**	0.8648	0.7017	0.5971	0.9758	0.9007
C:N Ratio	0.9321	0.1420	0.4922	0.1656	0.2651	0.3422	0.1344
N:S Ratio	**<0.0001**	**0.0003**	0.6597	0.8794	0.5821	0.1001	0.4377

Table 3.12. *S. alterniflora* plant metrics and nutrient concentrations by treatment (mean ± S.E.; N=4 unless otherwise noted) in greenhouse experiment #2. Treatments are NaCl (psu): SO_4^{-2} (mM). Different letters indicate significant differences among treatments.

Metric Mean ± S.E.	**(1) Trt:0,0 w/SC**	**(2) Trt: 0,0 w/SA**	**(3) Trt: 0,0 Alone**	**(4) Trt: 0,28 w/SC**	**(5) Trt: 0,28 w/SA**	**(6) Trt: 0,28 Alone**	**(7) Trt: 35, 0 w/SC**	**(8) Trt: 35, 0 w/SA**	**(9) Trt: 35,0 Alone**	**(10) Trt: 35,28 w/SC**	**(11) Trt: 35,28 w/SA**	**(12) Trt: 35,28 Alone**
Height (m/pot)	1.6 ± 0.4 ^{ab}	2.0 ± 0.2 ^{a}	1.3 ± 0.4 ^{abc}	1.3 ± 0.4 ^{abc}	2.3 ± 0.1 ^{a}	1.8 ± 0.4 ^{abc}	0.51 ± 0.10 ^{bc}	0.41± 0.05 ^{c}	0.93 ± 0.24 ^{abc}	0.84 ± 0.17 ^{abc}	0.41 ± 0.04 ^{c}	0.75 ± 0.11 ^{c}
Tiller (mm/pot)	15 ± 4.4 ^{ab}	15.3 ± 0.9 ^{a}	10.7 ± 3.5 ^{ab}	10.6 ± 4.0 ^{ab}	19.5 ± 1.8 ^{a}	15.2 ± 4.1 ^{ab}	4.5 ± 0.8 ^{b}	3.5 ± 0.6 ^{b}	5 ± 1 ^{b}	8 ± 1.4 ^{ab}	3 ± 0.4 ^{b}	5 ± 1.22 ^{b}
No. Leaves/ pot	33.7 ±7.4 ^{abc}	7.2 ± 3.3 ^{bc}	26.5 ± 9.0 ^{abc}	25.6 ± 9.5 ^{bc}	44.2 ± 0.4 ^{c}	36 ± 8.7 ^{abc}	9.2 ± 2.7 ^{abc}	6.7 ± 1.4 ^{ab}	9 ± 2.8 ^{ab}	12.5 ± 2.2 ^{abc}	7.2 ± 1.3 ^{a}	10 ± 2.2 ^{ab}
Photosynthetic Area (cm^2/pot)	898 ± 290 ^{ab}	933 ± 50.4 ^{b}	859 ± 355 ^{ab}	710 ± 317 ^{ab}	1043 ± 183 ^{ab}	1241 ± 249 ^{a}	208 ± 9 ^{c}	107 ± 15 ^{c}	176 ± 56 ^{c}	196 ± 58 ^{c}	147^{c} n=1	328 ± 11 ^{c}
Above Bio (g/pot)	4.4 ± 1.8 ^{ab}	4.4 ± 0.3 ^{ab}	3.3 ± 1.6 ^{ab}	1.7 ± 0.8 ^{ab}	6.7 ± 1.4 ^{a}	4.8 ± 1.3 ^{ab}	1.0 ± 0.4 ^{b}	1.6 ± 1.2 ^{b}	1.3 ± 0.2 ^{b}	1.1 ± 0.2 ^{b}	0.78 ± 0.26 ^{b}	1.6 ± 0.4 ^{ab}
Below Bio (g/pot)	4.1 ± 1.7 ^{a}	4.4 ± 1.5 ^{a}	2.6 ± 1.7 ^{a}	3.2 ± 2.4 ^{a}	3.8 ± 0.6 ^{a}	3.6 ± 1.8 ^{a}	0.54 ± 0.12 ^{a}	0.77 ± 0.43 ^{a}	0.55 ± 0.12 ^{a}	2.3 ± 0.9 ^{a}	0.42 ± 0.10 ^{a}	0.45± 0.19 ^{a}
Total Biomass (g/pot)	8.5 ± 3.5 ^{a}	8.8 ± 1.6 ^{a}	5.3 ± 2.9 ^{a}	4.5 ± 3.2 ^{a}	10.6 ± 2.0 ^{a}	7.5 ± 2.8 ^{a}	1.5 ± 0.5 ^{a}	2.4 ± 1.1 ^{a}	1.9 ± 0.2 ^{a}	3.5 ± 0.8 ^{a}	1.2 ± 0.3 ^{a}	1.9 ± 0.5 ^{a}
A:B ratio	1.4 ± 0.5 ^{a}	1.7 ± 0.9 ^{a}	2.3 ± 0.7 ^{a}	1.5 ± 1.1 ^{a}	1.7 ± 0.1 ^{a}	3.3 ± 2.0 ^{a}	1.7 ± 0.4 ^{a}	3.7 ± 2.9 ^{a}	3.0 ± 0.7 ^{a}	0.76 ± 0.25 ^{a}	1.9 ± 0.8 ^{a}	3.1 ± 0.5 ^{a}
No. Plnts/pot	5.7 ± 2.2 ^{a}	4.5 ± 1.8 ^{ab}	6.2 ± 2.7 ^{a}	2.2 ± 1.1 ^{abc}	10.5 ± 1.7 ^{a}	7.5 ± 2.4 ^{a}	1.2 ± 0.2 ^{bc}	1.2 ± 0.2 ^{bc}	1 ^{bc} n=1	2 ± 0.4 ^{c}	1 ^{c} n=1	1.5 ± 0.2 ^{c}
% Nitrogen	2.2 ± 0.2 ^{a}	2.6 ± 0.1 ^{a}	2.7 ± 0.2 ^{a}	2.4 ± 0.1 ^{a}	2.6 ± 0.3 ^{a}	2.8 ± 0.1 ^{a}	2.3 ± 0.1 ^{a}	2.2 ± 0.1 ^{a}	2.6 ± 0.4 ^{a}	3.1 ± 0.3 ^{a}	1.6 ± 0.8 ^{a}	2.1 ± 0.1 ^{a}
% Carbon	44.1 ± 0.9 ^{a}	43.1 ± 0.5 ^{a}	44.6 ± 1.3 ^{a}	43.5 ± 0.7 ^{a}	43.4 ± 0.3 ^{a}	43.5 ± 0.1 ^{a}	43.9 ± 0.2 ^{a}	42.1 ± 1.1 ^{a}	43.8 ± 0.5 ^{a}	43.6 ± 0.3 ^{a}	28.0 ± 13.9 ^{a}	42.1 ± 0.1 ^{a}
% Sulfur	0.37 ± 0.12 ^{b}	0.5 ± 0.1 ^{abc}	0.5 ± 0.1 ^{abc}	0.6 ± 0.1 ^{ab}	0.61 ± 0.09 ^{ab}	0.66 ± 0.04 ^{ab}	0.15 ± 0.02 ^{d}	0.17 ± 0.04 ^{d}	0.13 ± 0.02 ^{d}	0.35 ± 0.03 ^{c}	0.25 ± 0.15 ^{c}	0.35 ± 0.09 ^{c}
C:N	23.1 ± 2.3 ^{a}	18.9 ± 0.8 ^{a}	19.1 ± 1.5 ^{a}	20.9 ± 1.2 ^{a}	19.9 ± 3.0 ^{a}	18.0 ± 0.8 ^{a}	21.9 ± 0.5 ^{a}	22.5 ± 2.2 ^{a}	20.5 ± 3.2 ^{a}	16.8 ± 1.7 ^{a}	13.6 ± 5.9 ^{a}	23.9 ± 2.2 ^{a}
N:S	18.4 ± 7.0 ^{abc}	12.8 ± 2.3 ^{bc}	13.1 ± 2.5 ^{bc}	11.3 ± 3.6 ^{c}	10.4 ± 2.0 ^{bc}	10.0 ± 0.9 ^{c}	36.8 ± 5.8 ^{a}	29.3 ± 2.7 ^{ab}	47.0 ± 8.1 ^{a}	20.0 ± 0.8 ^{abc}	19.0 ± 4.1 ^{abc}	16.2 ± 3.1 ^{bc}

Table 3.13. ANOVA table for *S. cynosuroides* plant parameters from greenhouse experiment #2. Significant results ($p < 0.05$) are indicated in boldface.

Growth Characteristic	Sodium	Sulfate	Neighbor	Sodium x Neighbor	Sulfate x Neighbor	Sodium x Sulfate	Sodium x Sulfate x Neighbor
Height	0.0541	0.8025	**0.0051**	0.2753	0.4796	0.1602	0.3512
Tiller	**0.0110**	0.7225	0.2691	0.4644	0.7306	0.1549	0.3413
No. Leaves	**0.0054**	0.4946	0.9179	0.2643	0.3303	0.1932	**0.0220**
Photosynthetic Area	0.6856	0.7569	0.8194	0.6404	0.7846	0.4302	**0.0355**
Aboveground Biomass	**<0.0001**	0.2329	0.0575	0.2927	0.4244	0.1464	0.5959
Belowground Biomass	**0.0008**	**0.0410**	0.3353	0.6936	0.1940	0.1308	0.8416
Total Biomass	**0.0009**	**0.0371**	0.1054	0.9471	0.6163	0.1673	0.4560
Above:Belowground Biomass	0.8212	0.1418	0.1893	0.0832	0.6953	0.3570	0.4249
Number of plants	**0.0004**	0.2971	0.2795	0.7946	0.6733	0.2597	0.7667
% N	**0.0181**	0.2646	0.2447	0.0563	0.8400	0.8464	0.4597
% C	0.5637	**0.0425**	0.5881	0.4138	0.3974	0.0758	0.1609
% S	**<0.0001**	**0.0015**	0.6086	0.1846	0.2164	0.9804	0.1312
C:N Ratio	**0.0060**	0.4899	0.0977	**0.0310**	0.9060	0.7920	0.2376
N:S Ratio	**<0.0001**	**0.0013**	0.1338	0.5122	0.0625	0.2308	0.0914

Table 3.14. *S. cynosuroides* plant metrics and nutrient concentration by treatments (mean ± S.E.; N=4 unless otherwise noted) in greenhouse experiment #2. Treatments are NaCl (psu): SO_4^{-2} (mM). Different letters indicate significant differences among treatments.

Metric Mean ± S.E.	**(1) Trt:0,0 w/SA**	**(2) Trt: 0,0 w/SC**	**(3) Trt: 0,0 Alone**	**(4) Trt: 0,28 w/SA**	**(5) Trt: 0,28 w/SC**	**(6) Trt: 0,28 Alone**	**(7) Trt: 35, 0 w/SA**	**(8) Trt: 35, 0 w/SC**	**(9) Trt: 35,0 Alone**	**(10) Trt: 35,28 w/SA**	**(11) Trt: 35,28 w/SC**	**(12) Trt: 35,28 Alone**
Height (m/pot)	3.3 ± 0.6 ^{a}	1.9 ± 0.6 ^{ab}	1.2 ± 0.3 ^{b}	2.8 ± 0.4 ^{ab}	2.1 ± 0.4 ^{ab}	2.0 ± 0.5 ^{ab}	1.7 ± 0.3 ^{ab}	1.5 ± 0.1 ^{ab}	1.2 ± 0.2 ^{ab}	1.2 ± 0.3 ^{b}	1.3 ± 0.7 ^{ab}	0.93 ± 0.15 ^{ab}
Tiller (mm/pot)	19.2 ± 2.0 ^{a}	12 ± 3.1 ^{a}	9 ± 2.4 ^{a}	17.2 ± 2.4 ^{a}	16.2 ± 3.3 ^{a}	15.5 ± 4.6 ^{a}	9.7 ± 2.5 ^{a}	10 ± 0.7 ^{a}	9 ± 3.1 ^{a}	9 ± 2.1 ^{a}	13.6 ± 5.7 ^{a}	5.2 ± 1.1 ^{a}
No. Leaves/ pot	33.0 ± 2.3 ^{ab}	18.7 ± 5.6 ^{ab}	14.5 ± 3.6 ^{ab}	29.2 ± 4.0 ^{ab}	23.2 ± 4.3 ^{ab}	23.2 ± 6.9 ^{a}	17 ± 4.3 ^{b}	15.2 ± 0.8 ^{ab}	12.7 ± 4.0 ^{ab}	9.7 ± 3.4 ^{ab}	20.6 ± 8.4 ^{ab}	7.5 ± 1.5 ^{b}
Photosynthetic Area (cm^2/pot)	1204 ± 257 ^{a}	587 ± 205 ^{a}	313 ± 95 ^{a}	876 ± 171 ^{a}	577 ± 162 ^{a}	900 ± 151 ^{a}	483 ± 85 ^{a}	355 ± 42 ^{a}	358 ± 96.8 ^{a}	251 ± 165 ^{a}	443 ± 194.5 ^{a}	197 ± 48 ^{a}
Above Bio (g/pot)	9.3 ± 1.8 ^{a}	4.1 ± 2.4 ^{ab}	3.8 ± 0.4 ^{ab}	6.7 ± 1.2 ^{ab}	5.6 ± 1.7 ^{ab}	4.7 ± 1.5 ^{ab}	3.5 ± 0.3 ^{ab}	2.8 ± 0.3 ^{ab}	2.2 ± 0.7 ^{b}	1.5 ± 0.6 ^{b}	1.4 ± 0.5 ^{b}	1.5 ± 0.3 ^{b}
Below Bio (g/pot)	10.7 ± 3.8 ^{a}	5.7 ± 1.9 ^{a}	4.9 ± 1.7 ^{a}	5.2 ± 1.4 ^{a}	6.8 ± 2.1 ^{a}	5.9 ± 1.9 ^{a}	7.1 ± 1.2 ^{a}	3.1 ± 0.7 ^{a}	2.3 ± 0.9 ^{a}	1.4 ± 0.5 ^{a}	1.6 ± 0.5 ^{a}	1.5 ± 0.6 ^{a}
Total Biomass (g/pot)	20.1 ± 5.5 ^{a}	9.8 ± 4.3 ^{ab}	8.7 ± 1.9 ^{ab}	12.0 ± 1.4 ^{ab}	12.4 ± 3.8 ^{ab}	9.1 ± 3.5 ^{ab}	10.6 ± 1.4 ^{ab}	5.9 ± 1.0 ^{ab}	4.6 ± 1.6 ^{ab}	3.0 ± 1.2 ^{ab}	1.2 ± 0.3 ^{b}	3.1 ± 1.0 ^{ab}
A:B ratio	1.3 ± 0.5 ^{a}	0.55 ± 0.20 ^{a}	1.0 ± 0.2 ^{a}	3.2 ± 2.3 ^{a}	0.82 ± 0.04 ^{a}	1.2 ± 0.3 ^{a}	0.53 ± 0.09 ^{a}	0.97 ± 0.12 ^{a}	2.2 ± 0.2 ^{a}	1.0 ± 0.2 ^{a}	0.87 ± 0.22 ^{a}	1.0 ± 0.1 ^{a}
No. Plnts/pot	5 ± 0.4 ^{a}	4.7 ± 1.2 ^{ab}	4.2 ± 1.1 ^{a}	5.2 ± 0.7 ^{a}	4.2 ± 0.6 ^{a}	4.5 ± 1.3 ^{a}	2.7 ± 0.7 ^{ab}	3.7 ± 0.4 ^{ab}	2.5 ± 0.6 ^{ab}	2.7 ± 0.8 ^{ab}	2 ± 0.4 ^{ab}	1.7 ± 0.2 ^{b}
% Nitrogen	1.9 ± 0.1 ^{a}	1.7 ± 0.3 ^{a}	2.1 ± 0.1 ^{a}	1.6 ± 0.02 ^{a}	1.5 ± 0.1 ^{a}	2.1 ± 0.1 ^{a}	1.6 ± 0.0 ^{a}	1.5 ± 0.1 ^{a}	1.6 ± 0.1 ^{a}	1.5 ± 0.2 ^{a}	1.4 ± 0..2 ^{a}	1.4 ± 0.1 ^{a}
% Carbon	43.7 ± 0.1 ^{a}	43.2 ± 0.4 ^{a}	43.4 ± 0.2 ^{a}	43.2 ± 0.1 ^{a}	43.8 ± 0.1 ^{a}	42.6 ± 0.3 ^{a}	45.1 ± 0.7 ^{a}	45.1 ± 0.2 ^{a}	43.7 ± 0.3 ^{a}	41.7 ±. 1.5 ^{a}	37.2 ± 5.8 ^{a}	43.7 ± 0.3 ^{a}
% Sulfur	0.45 ± 0.03 ^{ab}	0.48 ± 0.05 ^{ab}	0.53 ± 0.06 ^{ab}	0.63 ± 0.06 ^{a}	0.53 ± 0.03 ^{a}	0.60 ± 0.06 ^{a}	0.21 ± 0.01 ^{c}	0.12 ± 0.01 ^{c}	0.18 ± 0.03 ^{c}	0.30 ± 0.08 ^{bc}	0.33 ± 0.08 ^{bc}	0.16 ± 0.05 ^{c}
C:N	27.2 ± 1.8 ^{a}	30.9 ± 5.0 ^{a}	24.3 ± 1.7 ^{a}	29.8 ± 0.3 ^{a}	33.8 ± 2.0 ^{a}	23.0 ± 0.5 ^{a}	32.1 ± 1.1 ^{a}	33.3 ± 1.5 ^{a}	30.6 ± 1.8 ^{a}	31.7 ± 3.3 ^{a}	30.8 ± 0.5 ^{a}	35.3 ± 4.5 ^{a}
N:S	9.7 ± 0.3 ^{acd}	8.1 ± 1.0 ^{ad}	9.3 ± 1.5 ^{acd}	6.2 ± 0.5 ^{a}	6.5 ± 0.3 ^{a}	8.4 ± 0.9 ^{ad}	18.4 ± 1.1 ^{bcd}	31.2 ± 2.3 ^{b}	21.8 ± 1.3 ^{b}	14.0 ± 4.1 ^{abcd}	11.7 ± 3.6 ^{abc}	23.3 ± 4.5 ^{bc}

Table 3.15. *S. alterniflora* (SA) and *S. cynosuroides* (SC) macronutrient values (mean ± S.E.) from both greenhouse experiments and a field experiment (see Chapter 2); treatments are NaCl:SO_4^{-2} and N=3 for each species. Different letters indicate significant differences among treatments within either the greenhouse or field experiments. Highest significance level of t-test comparisons between *S. alterniflora* and *S. cynosuroides* tissue concentrations in each experiment is indicated by asterisks; *-p<0.05; **-p<0.01; ***-p<0.001. (ns= not significant and nd=not detectable). Significant ANOVA results (p<0.05) are indicated in boldface.

Nutrient	GH1 5 psu: 4 mM	GH2 0 psu: 0 mM	GH2 0 psu: 28 mM	GH2 35 psu: 0 mM	GH2 35 psu: 28 mM	SA Zone	SC Zone	p
Al	ns	**	ns	ns	*	ns	ns	
SA	18.1 ± 0.7	200 ± 16	161 ± 80	140 ± 47	177 ± 7	1542 ± 433	3047 ± 1708	0.0669
SC	***23.3 ± 13.2*** [a]	78.8 ± 21.3 [a]	47.3 ± 7.6 [a]	112 ± 48 [a]	49.2 ± 14.0 [a]	2051 ± 78 [b]	110 ± 25 [b]	**<0.0001**
Ba		ns						
SA	nd	41.6 ± 6.0	12.9; n=1	nd	15.0 n=1	nd	nd	.
SC	nd	32.3 ± 1.2	nd	nd	nd	nd	nd	.
Ca	*	ns	ns	*	ns	**	*	
SA	1397 ± 315 [bd]	3531 ± 352 [c]	2609 ± 179 [abc]	1234 ± 179 [d]	1654 ± 474 [ab]	3385 ± 343 [abc]	3190 ± 581 [abc]	**0.0230**
SC	3605 ± 446 [a]	4050 ± 272 [a]	3180 ± 326 [a]	1840 ± 115 [b]	1167 ± 298 [b]	930 ± 156 [b]	1140 ± 136 [b]	**<0.0001**
Fe	**	**	ns	ns	*	ns	ns	
SA	79.6 ± 5.9 [a]	182 ± 4 [a]	130 ± 44 [a]	129 ± 23 [a]	148 ± 11 [a]	939 ± 240 [a]	1861 ± 884 [a]	**0.0298**
SC	50.9 ± 1.6 [c]	74.4 ± 11.6 [c]	59.9 ± 4.4 [c]	66.9 ± 28.7 [c]	77.8 ± 16.5 [c]	1129 ± 43 [a]	268 ± 28 [b]	**<0.0001**
K	ns	ns	*	*	ns	*	***	
SA	16555 ± 3235 [b]	35860 ± 6905 [a]	20203 ± 1935 [b]	8809 ± 754 [b]	7373 ± 1258 [b]	9470 ± 1682 [b]	10031 ± 171 [b]	**0.0002**
SC	11585 ± 1469 [b]	17880 ± 1131 [a]	1487 ±264 [ab]	4615 ± 695 [cd]	6634 ± 292 [c]	3519 ± 226 [cd]	1374 ± 385 [d]	**<0.0001**
Mg	*	**	ns	Ns	ns	**	**	
SA	496 ± 17 [ac]	1667 ± 43 [a]	870 ± 130 [a]	531 ± 63 [c]	682 ± 257 [ac]	4774 ± 421 [b]	4391 ± 256 [b]	**<0.0001**
SC	742 ± 45 [b]	2081 ± 36 [a]	1122 ± 83 [b]	587 ± 82 [b]	696 ± 88 [b]	2449 ± 156 [a]	1057 ± 194 [b]	**<0.0001**
Mn	ns	*	*	*	ns	**	ns	
SA	228 ± 79 [c]	1164 ± 130 [a]	837 ± 100 [ab]	217 ± 38 [c]	345 ± 20 [c]	257 ± 16 [c]	499 ± 117 [bc]	**<0.0001**
SC	463 ± 118 [b]	1798 ± 146 [a]	1445 ± 165 [a]	508 ± 53 [b]	387 ± 101 [b]	87.2 ± 11.5 [b]	349 ± 40 [b]	**<0.0001**
Na	*	*	**	*	**	**	**	
SA	11825 ± 305 [abc]	1957 ± 206 [b]	6941 ± 584 [a]	10548 ± 635 [a]	16496 ± 1928 [c]	9551 ± 928 [a]	6581 ± 941 [ab]	**<0.0001**
SC	5906 ± 1178 [ab]	932 ± 204 [d]	3395 ± 307 [bcd]	6050 ± 1066 [ab]	7251 ± 562 [a]	4290 ± 530 [abc]	2043 ± 173 [dc]	**<0.0001**
P	ns	**	**	ns	ns	*	*	
SA	4150 ± 851 [ac]	6164 ± 565 [ab]	6829 ± 512 [b]	2852 ± 276 [cd]	2612 ± 119 [cd]	1667 ± 254 [d]	2335 ± 422 [cd]	**<0.0001**
SC	3143 ± 647 [a]	3332 ± 151 [a]	3486 ± 307 [a]	2546 ± 264 [a]	1930 ± 428 [ab]	720 ± 85 [b]	666 ± 98 [b]	**<0.0001**
Si	ns	*	ns	*	*	Ns	*	
SA	1730 ± 187	811 ± 108	1260 ± 789	1819 ± 329	1907 ± 285	2477 ± 296	2865 ± 863	0.1645
SC	1737 ± 759 [a]	447 ± 21 [a]	329 ± 30 [a]	339 ± 144 [a]	836 ± 280 [a]	1687 ± 17 [a]	428 ± 40 [a]	**0.0146**
Sr		ns				**		
SA	nd	17.3 ± 0.8 [b]	nd	nd	nd	51.6 ± 4.0 [a]	36.0 ± 4.1 [ab]	**0.0055**
SC	nd	15.2 ± 0.3 [a]	nd	nd	nd	15.6 ± 1.5 [a]	nd	0.7499
Zn			*	**	*			
SA	37.7 ± 9.3	105 ± 20	74.8 ± 9.2	93.1 ± 4.3	86.6 ± 15.7	nd	nd	.
SC	44.6 ± 6.6	54.1 ± 13.9	38.4 ± 1.5	26.5 ± 6.0	27.4 ± 4.8	nd	nd	.

Table 3.16. *Spartina alterniflora* and *S. cynosuroides* (SC) elemental composition (% for N and S, ppm for all other nutrients). *S. alterniflora* values reported are for "tall" form plants unless otherwise noted. *S. cynosuroides* values are only available for % N, K, Na, and P (noted with SC in text).

Source	N	S	Al	Ba	Ca	Fe	K	Mg	Mn	Na	P	Si	Sr	Zn
Broome et al. (1975) NC	0.77-0.98	0.4-0.55	--	--	3200	701.6	7700	2900-4600	30.6	25000	1000-1200	--	--	--
Gallagher 1975 (GA)	0.7	--	--	--	2100	--	6300	4300	55	--	1400	--	24	13
Patrick & Delaune 1976	0.7-0.9	--	--	--	--	--	--	--	--	--	800-1500	--	--	--
Chalmers 1979 (GA)	1.4	--	--	--	--	--	--	--	--	--	--	--	--	--
Linthurst 1979	0.78-0.88	0.21-0.49	--	--	1500-3000	--	10200-14300	2400-3000	--	--	1100-1200	--	--	~20
Mendelssohn 1979	1.69	--	--	--	--	--	--	--	--	--	--	--	--	--
Gallagher et al. 1980	1.0	--	--	--	1500	--	10000	3300	--	--	1700	--	--	--
Smart & Barko[a] 1980	0.66-0.80	--	--	--	--	--	5700 (marine) 6100 (fresh)	--	--	7700 (marine) 7000 (fresh)	400-500	--	--	--
Linthurst & Seneca 1981	1.07	0.35	--	--	3000	--	12800	3500	--	--	2000	--	--	--
Carlson & Forrest 1982	--	0.13	--	--	--	--	--	--	--	--	--	--	--	--
Hopkinson & Schubauer 1983	1.05	--	--	--	--	--	--	--	--	--	--	--	--	--
Hopkinson & Schubauer 1984	~0.66 (SC)	--	--	--	--	--	--	--	--	--	--	--	--	--
Valiela 1984	1.4	--	--	--	--	--	--	--	--	--	--	--	--	--
Broome et al. 1986	0.7	0.5	--	--	2500	--	7800	4600	--	900		--	--	--
Hackney & de la Cruz 1986 (MS)	~0.4-1.0 (SC)	--	--	--	--	--	--	--	--	700 (SC)	--	--	--	--
Ornes & Kaplan 1989 (GA)	1.32	0.32	--	--	2600	--	12300	3500	--	--	1900	--	--	--

Source	N	S	Al	Ba	Ca	Fe	K	Mg	Mn	Na	P	Si	Sr	Zn
Beale & Long 1991 (United Kingdom)	0.3 (SC)	--	--	--	--	--	1000 (SC)	--	--	--	400 (SC)	--	--	--
Bradley & Morris[b] 1991	--	0.32 0.35	--	--	2004 1603	--	14076 8602	4132.7 8508.5	--	45520 117708	--	--	--	--
Stribling & Cornwell 2001 (MD)	~0.75-3.07 (freshwater) ~0.05-0.2 (saltwater)	~0.33-0.53 (freshwater) ~0.24-0.69 (saltwater)	--	--	--	--	--	--	--	300-3800 (freshwater) 500-2000 (saltwater)	--	--	--	--
Ornes et al 1998	--	--	~800-4000	--	--	320-870	--	--	25-80	--	--	--	--	8-16
DeLaune et al 1983	--	--	--	--	--	--	--	--	--	--	--	--	--	13

[a] Critical nutrient levels of N and P.

[b] Values for plants grown in salinity treatments of 10 and 40 g/dm^3 respectively.

Greenhouse Experiment #1: Single species with NaCl and SO_4^{-2} additions

Design: 2 species * 8 treatments * 7 replicates

Treatments:
(I= ionic strength)

		Salinity (psu)		
		0	5	35
Sulfate (mM)	0	I= 0	I= 0.072	I= 0.510
	4	I= 0.008	I= 0.081	I= 0.518
	28	**X**	I= 0.129	I= 0.566

'X' denotes the treatment that was not used due to an ionic strength (I=0.056) that was too similar to those found in treatment combinations with 5 psu.

Greenhouse Experiment #2: Species interactions with NaCl and SO_4^{-2} additions

Design: 2 species * 12 treatments * 4 replicates

Treatments:

		Salinity (psu)					
		0			35		
Sulfate (mM)	0	Opp	Con	Alone	Opp	Con	Alone
	28	Opp	Con	Alone	Opp	Con	Alone

Neighbor treatments included:

With opposite species (Opp) With conspecific species (Con) Alone

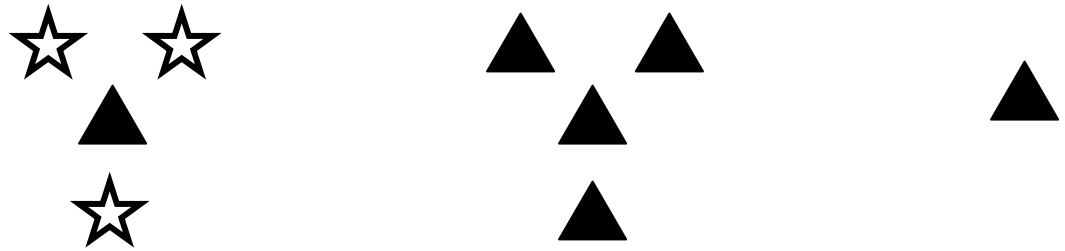

Figure 3.1. Greenhouse Experiment #1 and #2: Treatment matrices including ionic strengths for greenhouse experiment #1.

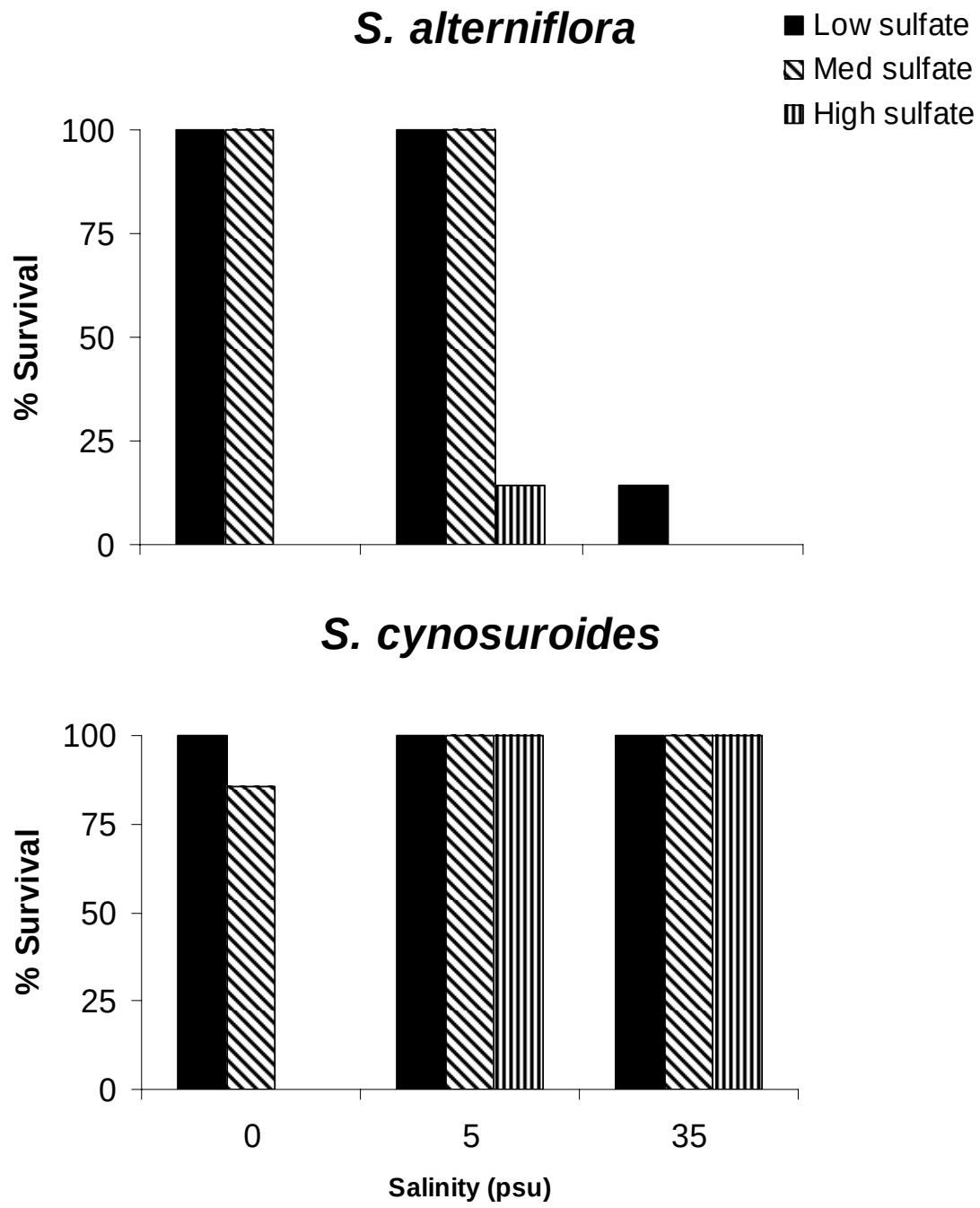

Figure 3.2. Greenhouse Experiment #1. *S. alterniflora* (top) and *S. cynosuroides* (bottom) target plant percent survival in manipulated salinity and sulfate treatments. Sulfate treatments are as follows: dark bars = low sulfate (0 mM SO_4^{-2}), diagonally striped bars = medium sulfate (4 mM SO_4^{-2}), and vertically striped bars= high sulfate (28 mM SO_4^{-2})

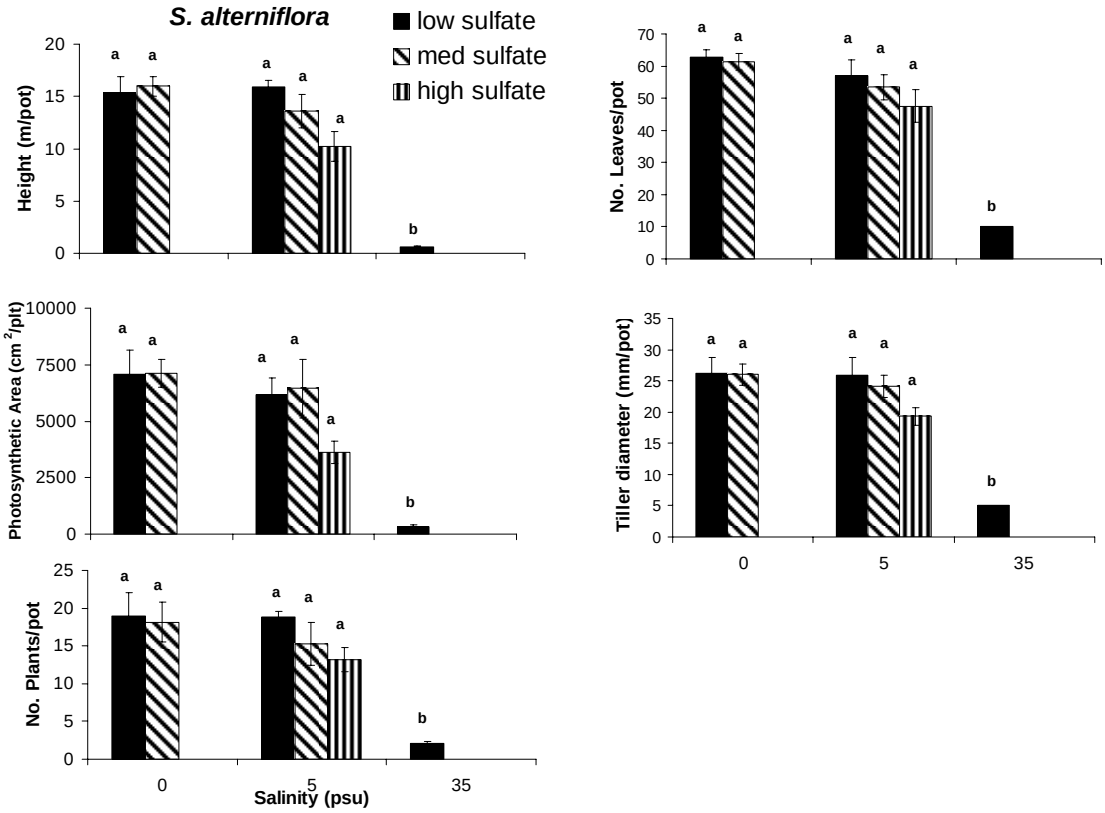

Figure 3.3. Greenhouse Experiment #1. *S. alterniflora* plant growth means (± S.E.) by salinity and sulfate treatments.

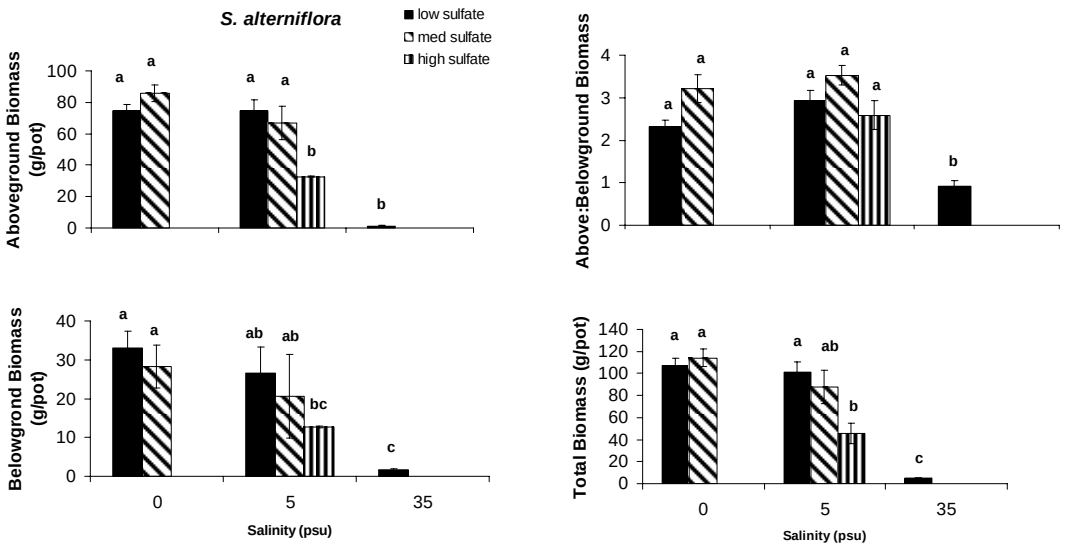

Figure 3.4. Greenhouse Experiment #1. *S. alterniflora* plant biomass means (± S.E.) by salinity and sulfate treatments.

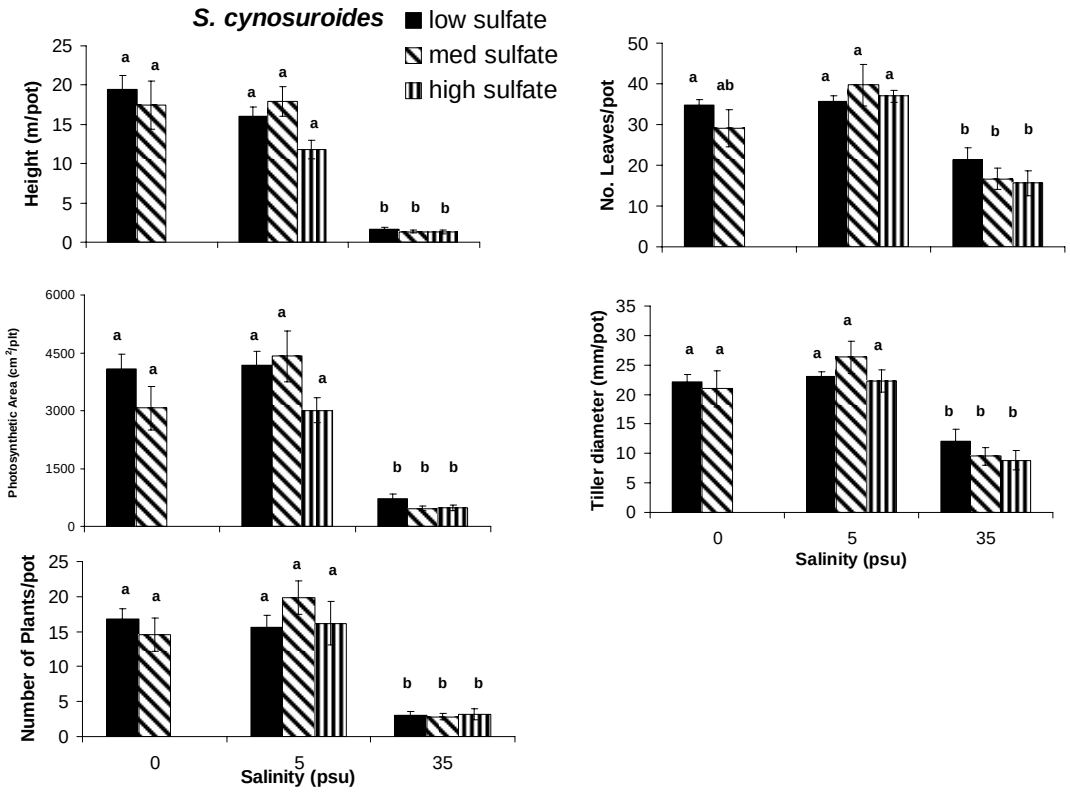

Figure 3.5. Greenhouse Experiment #1. *S. cynosuroides* plant growth (means ± S.E.) by salinity and sulfate treatments.

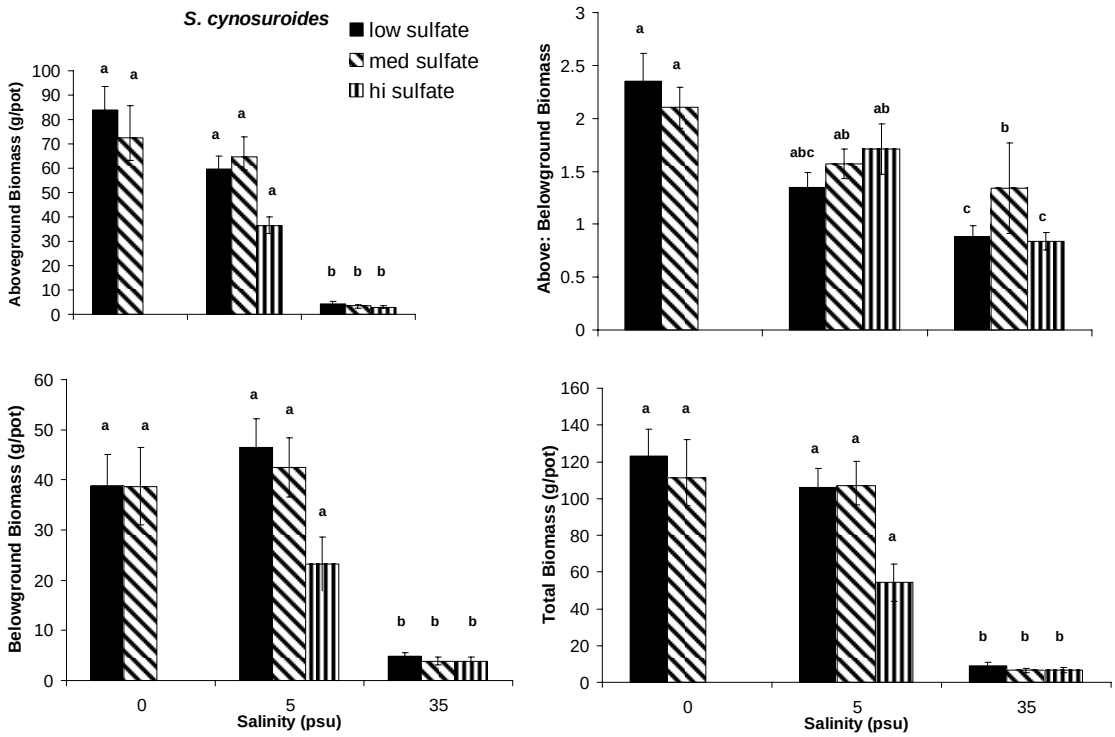

Figure 3.6. Greenhouse Experiment #1. *S. cynosuroides* plant biomass means (± S.E.) by salinity and sulfate treatments.

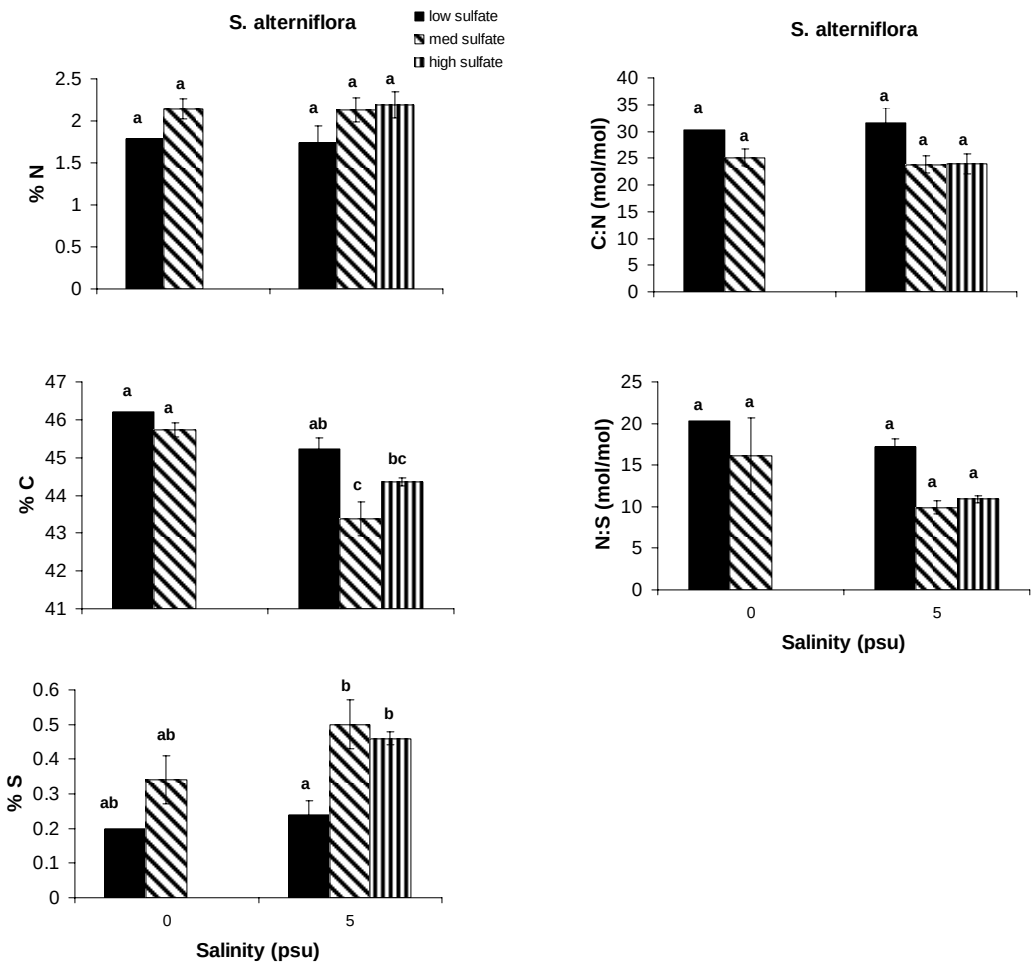

Figure 3.7. Greenhouse Experiment #1. *S. alterniflora* plant tissue nutrients (means ± S.E.) by salinity and sulfate treatments.

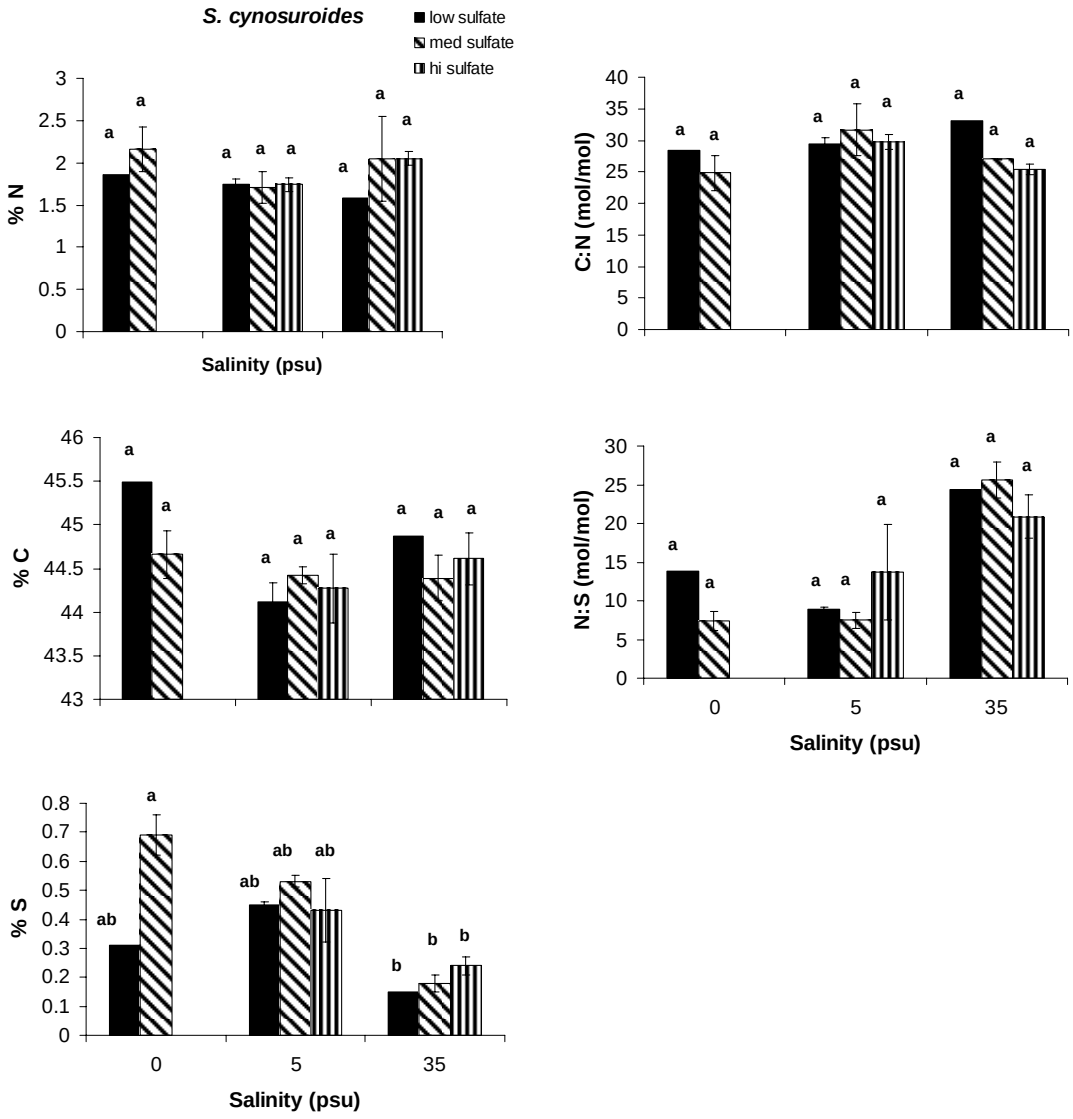

Figure 3.8. Greenhouse Experiment #1 *S. cynosuroides* plant tissue nutrients (means ± S.E.) by salinity and sulfate treatments.

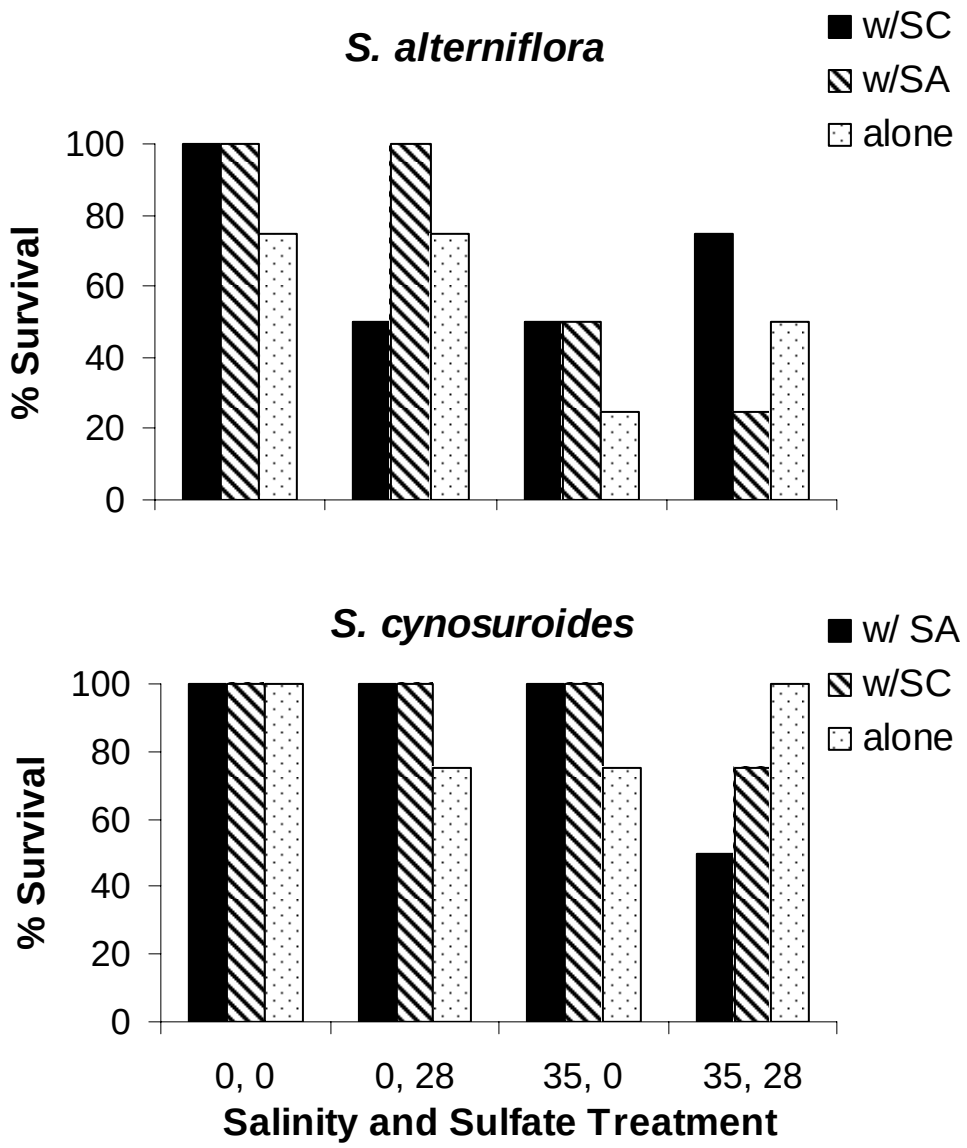

Figure 3.9. Greenhouse Experiment #2. *S. alterniflora* (top) and *S. cynosuroides* (bottom) target plant survival in salinity, sulfate, and neighbor treatments. Salinity and sulfate treatments are indicated on the x-axis (salinity in psu followed by mM SO_4^{-2}), and neighbor treatments are as follows: dark bars = opposite species neighbor, striped bars = with same species neighbor, and stippled bars = alone.

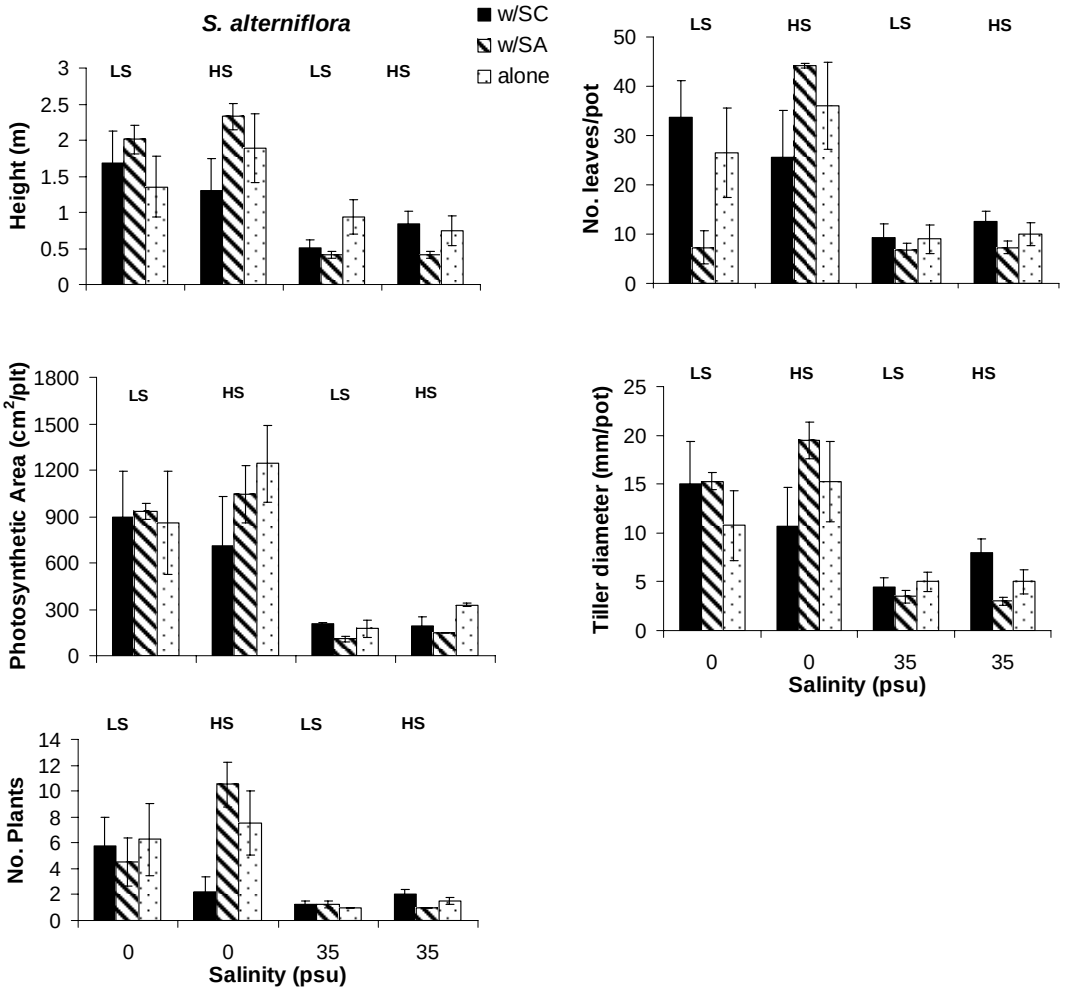

Figure 3.10. Greenhouse Experiment #2. *S. alterniflora* plant growth means (± S.E.) by salinity, sulfate and neighbor treatment. Low Sulfate (LS) and High Sulfate (HS) are labeled above the bars.

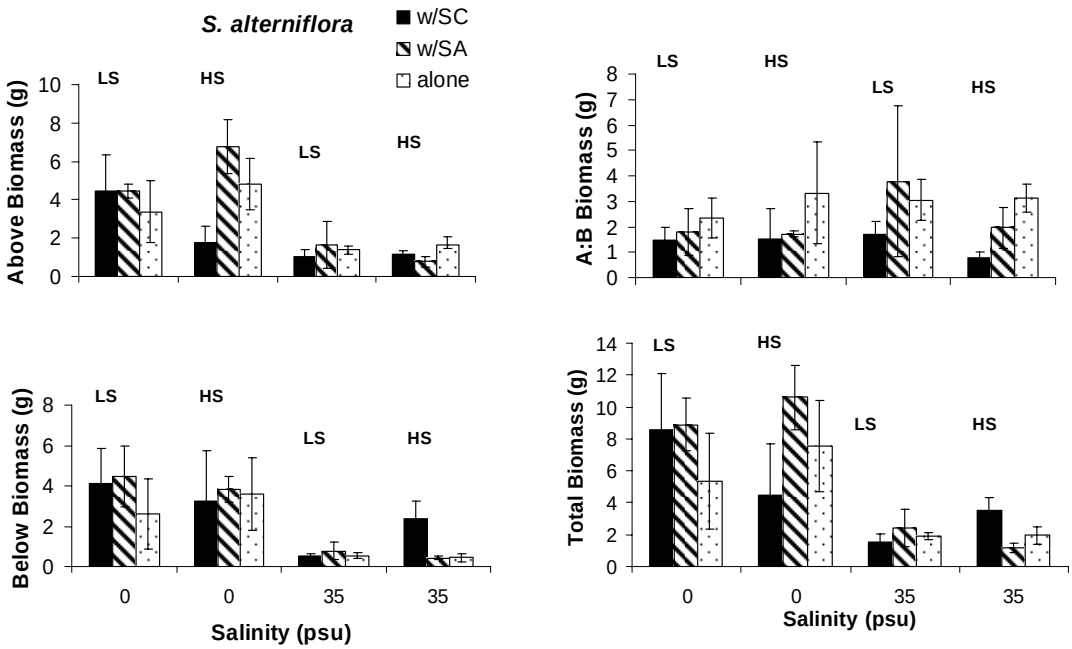

Figure 3.11. Greenhouse Experiment #2. *S. alterniflora* plant biomass means (± S.E.) by salinity, sulfate, and neighbor treatment. Low Sulfate (LS) and High Sulfate (HS) are labeled above the bars.

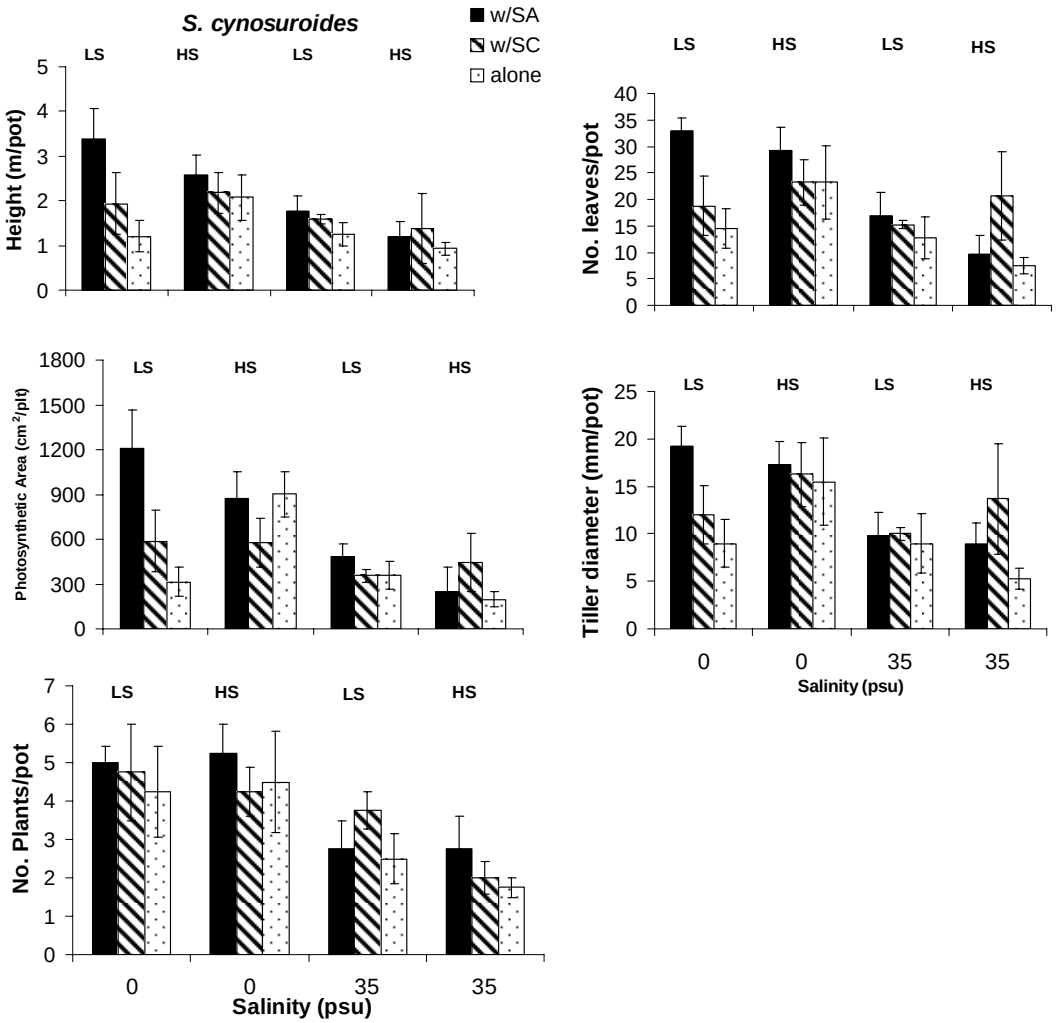

Figure 3.12. Greenhouse Experiment #2. *S. cynosuroides* plant growth means (± S.E.) by salinity, sulfate and neighbor treatment. Low sulfate (LS) and High Sulfate (HS) are labeled above the bars.

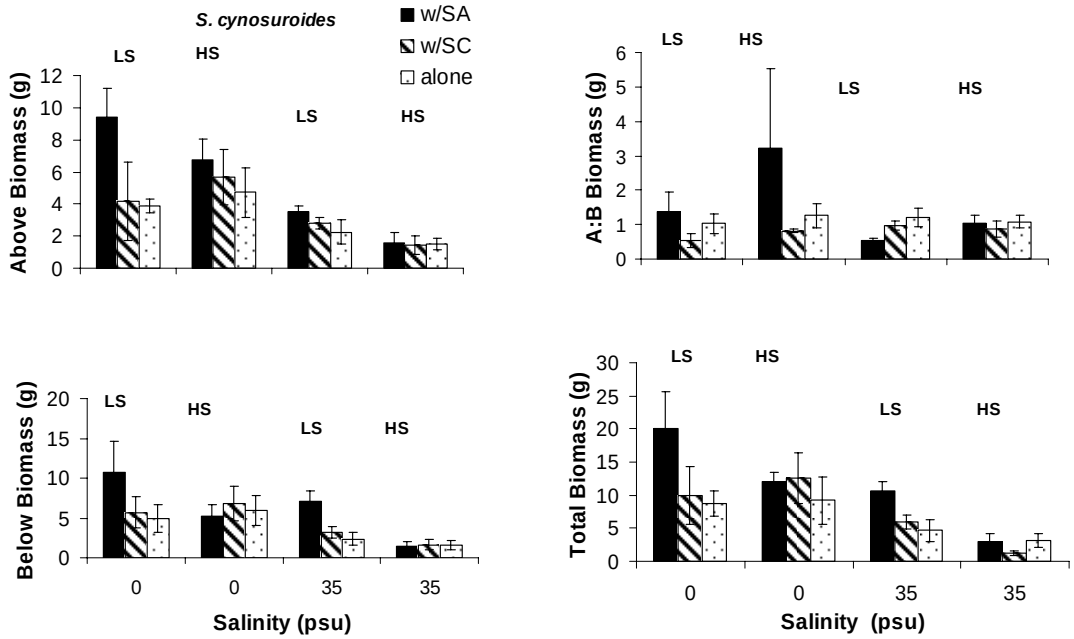

Figure 3.13. Greenhouse Experiment #2. *S. cynosuroides* plant biomass means (± S.E.) by salinity, sulfate, and neighbor treatment. Low sulfate (LS) and High Sulfate (HS) are labeled above the bars.

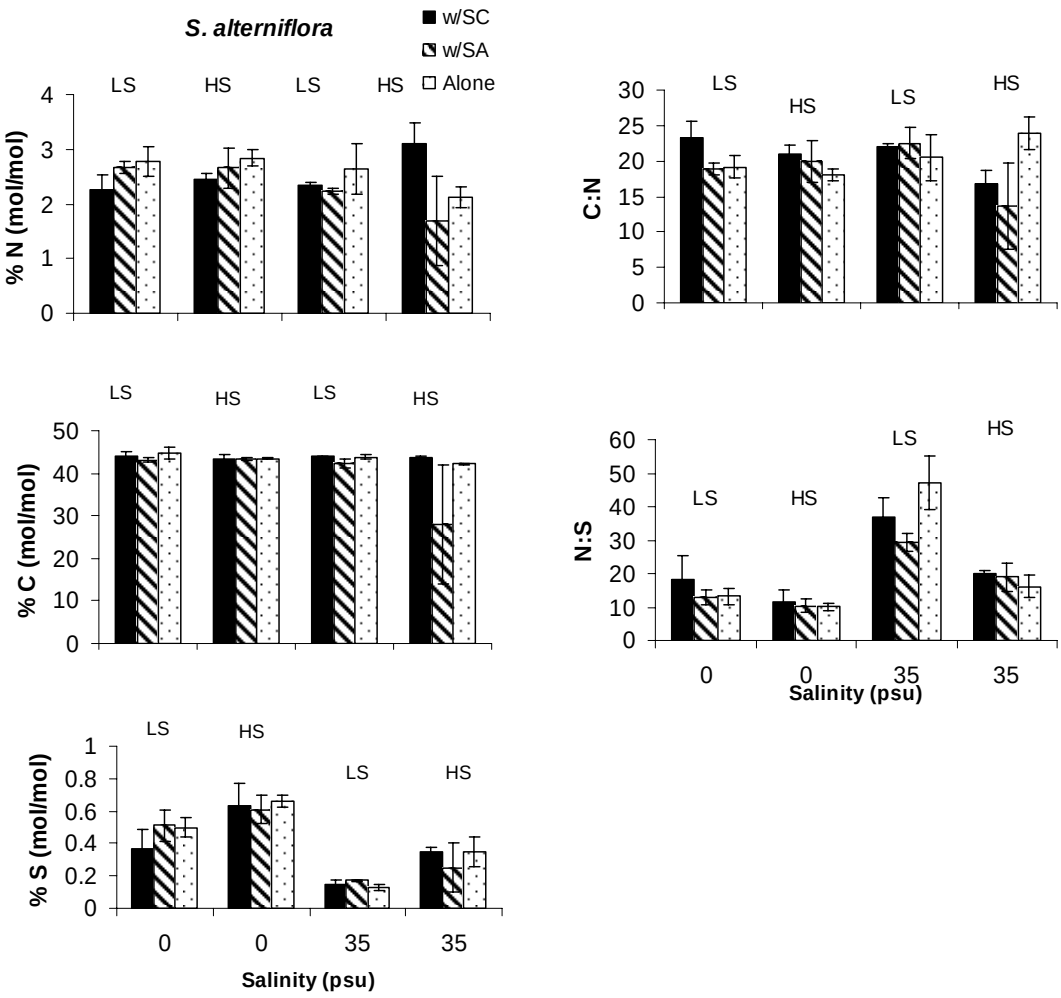

Figure 3.14. Greenhouse Experiment #2. *S. alterniflora* plant tissue nutrients (means ± S.E.) by salinity, sulfate and neighbor treatments. Low sulfate (LS) and High Sulfate (HS) are labeled above the bars.

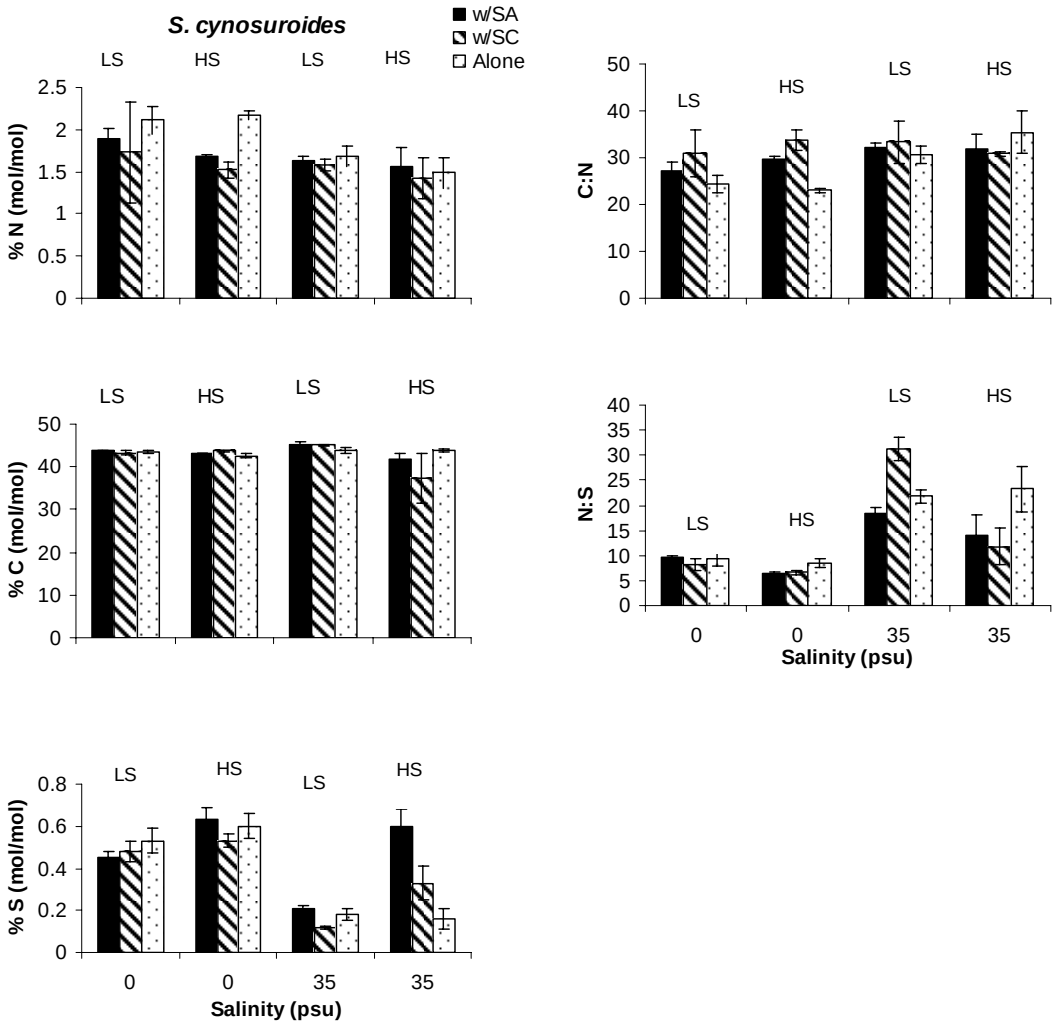

Figure 3.15. Greenhouse Experiment #2. *S. cynosuroides* plant tissue nutrients (means ± S.E.) by salinity, sulfate and neighbor treatments. Low sulfate (LS) and High Sulfate (HS) are labeled above the bars.

CHAPTER 4

THE RESPONSE OF *SPARTINA* SPECIES TO PROLONGED DROUGHT IN THE ALTAMAHA RIVER ESTUARY

Salinity is often considered a key predictor for the distribution of habitats and organisms along an estuary (Adams 1963, Odum 1988, Mitsch and Gosselink 1993). Temperate riverine estuaries are characterized by distinct vegetation along the salinity gradient, with salt marsh vegetation in the polyhaline range (>18 psu), brackish marsh plants in the oligo- and mesohaline ranges (0.5-18 psu), and tidal freshwater plants furthest upstream (<0.5 psu; Odum, 1988). In the southeastern United States different species of *Spartina* can be found along the longitudinal gradient of estuaries: *Spartina alterniflora* is found in the salt marshes and *S. cynosuroides* is found in fresher areas, although the two species overlap at intermediate salinities. This study focused on documenting changes in the distribution of both *Spartina* species along the length of the Altamaha River estuary, Georgia, over the course of a regional drought.

Spartina alterniflora Loisel (smooth cordgrass) is found in low, intertidal salt marshes throughout the east and Gulf coasts of the United States (Smart 1982, Mitsch and Gosselink 1993). *Spartina alterniflora* thrives in these environments because it is able to tolerate high salinities. At lower salinities (found typically at higher elevations in the marsh) it is displaced by competitively dominant marsh species, such as *S. patens* and *Juncus gerardi* (Bertness and Ellison 1987, Bertness 1991a, b). *Spartina* species have

developed a number of physiological mechanisms that allow them to be successful in high salt environments, including salt glands/salt secretion (Levering and Thompson 1971, Anderson 1974), osmotic adjustment (Cavalieri and Huang 1981), root excretion of salts (Smart and Barko 1980), and selective ion uptake (Flowers et al. 1977, Smart and Barko 1980, Smart 1982, Bradley and Morris 1991). *Spartina alterniflora* salt marsh communities are considered to be one of the world's most productive ecosystems (Odum 1971, Whittaker 1975, Howes et al. 1986, Mitsch and Gosselink 1993). Valiela (1995) estimated that, on average over its range, net primary production in marshes dominated by *S. alterniflora* was about 3,000 g C m^{-2} yr^{-1} whereas estimates of net primary production for *S. alterniflora* in Georgia marshes range from 1,100 to 7, 600 g C dry mass m^{-2} yr^{-1} (Schubauer and Hopkinson 1984).

In contrast to the available information concerning *S. alterniflora*, less is known about its brackish marsh counterpart, *S. cynosuroides*. *Spartina cynosuroides* (L.) Roth is typically found in irregularly-flooded salt, brackish and tidal fresh marshes from southern Massachusetts to Florida and Texas (Tiner 1993, Stuckey and Gould 2000). Plant height varies from 1-4 m and leaf blades are heavily serrated and can be up to 75 cm long with widths between 5 and 25 mm (Stuckey and Gould 2000). Studies using *S. cynosuroides* have been conducted in the United Kingdom where production rates were quantified (yields ~15-20 ton dry weight/ha) in order to assess whether this plant could be used as a source of biofuel (Potter et al. 1995). Schubauer and Hopkinson (1984) investigated *S. cynosuroides* production in a southeastern estuary and estimated that its net primary production rate was approximately 7,708 g C dry mass m^{-2} yr^{-1}. *Spartina cynosuroides* salinity tolerance is considered to be less than that of *S. alterniflora* but greater than that

of plants typically found in tidal freshwater marshes. In the Altamaha River, GA, Schubauer and Hopkinson (1984) found that interstitial salinities in a *S. cynosuroides* stand ranged from 2 to 14 psu over the course of the year. However, the actual controls of *S. cynosuroides* distribution in estuarine systems have not been explicitly explored (but see Chapter 2).

I investigated *Spartina* species distributions along the Altamaha River estuary in Georgia. The Altamaha River estuary is approximately 54 km in length with an average width of 1.04 km and an average depth of 4.0 m (Dame et al. 2000, Sheldon and Alber 2002). Tidal range is approximately 2-3 m. Under typical conditions, salinity at the mouth of the estuary is approximately 20 psu and it decreases to 0 psu 20 km upstream (Alber and Sheldon 1999). Our understanding of the distribution of vegetation along the length of the estuary is based on GIS mapping efforts (Higinbotham et al. 2004) that broadly delineated areas of salt marsh (up to 6 km from the mouth), brackish marsh (located along the creekbank from 6 to 16 km upriver) and freshwater marsh (upstream of 16 km). Ground-truthing of these categories found that areas classified as salt marsh were dominated by *S. alterniflora*, areas classified as brackish marsh had a mixture of both *S. alterniflora* and *S. cynosuroides* along the creekbank, and areas classified as freshwater marsh were dominated by *Zizania* and *Zizaniopsis* (Higinbotham et al. 2004). They also found that the division between salt and brackish marsh communities occurred where high tide salinities averaged 15 psu. Salinity values were calculated from data collected between 1995 and 1999 and applied to GIS maps based on aerial photographs taken in 1993.

Between 1999 and 2002 Georgia experienced a prolonged drought and freshwater inflow to the estuary decreased considerably. From 1968 to 1997, median discharge was 250 $m^3 s^{-1}$ (based on USGS data at Doctortown, corrected for the ungauged portion of the watershed) whereas during the drought period it decreased by half, to 124 $m^3 s^{-1}$ (Figure 4.1). The lowest annual median discharge occurred in 2000, when it was reduced to 107 $m^3 s^{-1}$. As a consequence, saltwater penetrated further upstream such that salinities as high as ~10 psu were recorded 16 km from the mouth in 2001 and sustained average salinities of approximately 3 psu were observed 20 km upriver in 2000 and 2001 (Georgia Coastal Ecosystems Long Term Ecological Research [GCE-LTER] Monitoring Data; (2000). Drought conditions were alleviated beginning in 2002-2003 as annual median discharge increased to 469 $m^3 s^{-1}$, and salinities 20 km upriver decreased to pre-drought conditions (average of 0.09 psu in 2003; GCE-LTER Monitoring Data). This extreme shift in environmental conditions along the Altamaha River estuary provided an opportunity to assess the response of both *S. alterniflora* and *S. cynosuroides* to increasing salinities. I was interested in documenting shifts in species distribution as well as evaluating the response of naturally mixed species stands over the course of the drought period.

Several investigators have documented vegetation shifts along the longitudinal axes of estuaries in response to changes in salinity. Perry and Hershner (1999) conducted a vegetation analysis of a tidal freshwater marsh on the Chesapeake Bay and found that *S. cynosuroides* and *Peltrandra virginica* co-dominated the habitat whereas 13 years earlier *P. virginica* had been the sole dominant. They suggested that this shift toward a community with more salt-tolerant species may have been a result of the influence of salt

water intruding upstream in this area (possibly due to sea level rise or subsidence). In the Savannah River, Pearlstine et al. (1993) reported that brackish marsh species replaced freshwater species following the construction of a tide gate that caused a 2 to 6 mile upstream displacement of salt water. They noted that within 2 months of the removal of the tide gate, salinities at downstream locations shifted from 12 psu to 4 psu. Visser et al. (2002) documented species shifts in a Louisiana estuary experiencing a 2-year drought, the largest of which was from oligohaline wiregrass (*S. patens* with *S. lancifolia*) to mesohaline wiregrass (*S. patens* dominated) communities.

There were two major questions that I was interested in addressing in this study; 1) Did *S. alterniflora* and *S. cynosuroides* distributions change over the course of the drought? and 2) How did mixed communities respond to changing conditions, and did plant interactions affect this response?

METHODS

Altamaha River Estuary Surveys

Vegetation surveys targeting creekbank communities along the Altamaha River estuary were conducted in September 2000, approximately one year after the onset of drought conditions, and again two years later in October 2002. Sites were established approximately every 2 km (2000, N=10) or 1 km (2002, N=14) between 3 and 19 km from the mouth of the estuary, with the majority of the stations located on the northern bank of the main channel (Figure 4.2). Upstream of 19 km the vegetation shifts to wild rice (*Zizaniopsis*), whereas downstream of 3 km, vegetation is generally a monoculture of *S. alterniflora*. Each site was located within 2 m of the riverbank and consisted of 3

quadrats (1.0 m^2) at least 2 m apart. Two randomly selected 0.25-m^2block areas within each quadrat were either assessed in the field or harvested and brought back to the laboratory for analysis. All *S. alterniflora* and *S. cynosuroides* shoots were identified and counted in each selected 0.25-m^2 block and various plant characteristics were measured. Measured parameters included height, number of leaves and tiller diameter on all plants and average leaf area of 5 randomly selected plants (3 leaf lengths and widths measured on each of the 5 plants). Total aboveground biomass was collected from the two blocks, harvested plants were washed free of debris, rinsed with deionized water, and dried at 60º C to constant weight. A subsample of leaves and stems from each site were ground in a Wiley Mill (40 µm mesh) and analyzed for C, N, and S content with a CE Elantech Flash Elemental Analyzer 1112 using a sulfanilamide standard. Ground plant tissues were not acidified before CNS analysis as preliminary runs did not show significant differences in C, N or S between acidified and un-acidified samples.

Data were analyzed using analysis of variance (ANOVA) with species, year, and salinity as fixed effects (Sokal and Rohlf 1995, SAS 2000). A significance level (alpha) of 0.05 was used for all statistical analyses unless otherwise stated. All data were tested for normality (Shapiro-Wilk test statistic) and homogeneity of variance (Bartlett test statistic) prior to analysis. Where necessary, data were appropriately transformed to meet these assumptions. Tukey's post-hoc tests were used to look at the effect of salinity on plant density and performance. T-tests were used to test for differences between years and species for both plant density and performance. Bonferonni adjusted p-values are p=0.001 for t-tests comparing differences between years and species for plant growth metrics.

Experimental Removals from Mixed Spartina Stands

Spartina alterniflora and *S. cynosuroides* coexist naturally in creekbank areas located approximately 6-16 km from the mouth of the Altamaha River estuary ((Higinbotham et al. in press). Within this mixed-species area a clipping experiment was established in June 2001, monitored for 2 growing seasons (June 2001, September 2001, March 2002, and October 2002), and harvested in October 2002. Clipped plots were located approximately 2 m from the creekbank, below the wrack line, and distributed along a 4 km length of the bankside (from 8-12 km) in 10 blocks (Figure 4.2). Each of the 10 blocks included 5 treatment plots that had been identified previously as either mixed *Spartina* stands or monotypic stands of each species. All plots were 0.7 m x 0.7 m (A=0.5 m^2) and each plot was separated from the next by at least 2 m. Treatments were as follows: 1) *S. cynosuroides* was removed from mixed *Spartina* stands (SA-sc), 2) *S. alterniflora* was removed from mixed *Spartina* stands (SC-sa), 3) mixed *Spartina* stands with neither *S. alterniflora* nor *S. cynosuroides* presence manipulated (control), 4) monotypic *S. alterniflora* stands with ½ of the *S. alterniflora* removed (½ SA), and 5) monotypic *S. cynosuroides* stands with ½ of the *S. cynosuroides* removed (½ SC). A sampling grid was used to separate the 0.5 m^2 area into 14 cm x 14 cm blocks (total=25). Individual species locations within mixed *Spartina* plots (treatments 1 and 2 only) were mapped over the course of both growing seasons in an effort to track species regrowth. These data were highly variable and were not useful for understanding *Spartina* regrowth patterns, so the results are not included here. Treatments, where either *S. alterniflora* or *S. cynosuroides* were removed from mixed *Spartina* stands (treatments 1 and 2, were maintained every 2 months over the course of both growing seasons.

Non-destructive measurements of plant performance were measured at the beginning and end of the experiment. Total number of plants was measured in all plots at the beginning, during plot maintenance, and at the end of the experiment. Plant height, tiller diameter, and number of leaves were recorded for all *S. alterniflora* plants in treatment 1 and all *S. cynosuroides* plants in treatment 2; 5 randomly chosen plants of each species were measured in the control plots (treatment 3); and 10 plants of each species were measured in each monotypic stand (treatments 4 and 5). Photosynthetic area was calculated for 3 randomly selected plants in each plot (3 leaf lengths and widths measured on each plant).

Final aboveground biomass was harvested in the fall of 2002. Plants were washed free of debris, rinsed with deionized water, and dried at 60º C to constant weight. A subsample of plants from each plot was ground in a Wiley Mill (40 μm mesh) and plant tissue carbon, nitrogen, and sulfur concentrations were analyzed as described above. In order to evaluate if plants located outside the treatment plots were influencing growth characteristics, there were two separate harvests in each plot: an inner harvest that consisted of plants located at the center of the plot (Area ~ 0.25 m^2) and an outer harvest that consisted of plants growing around the outer edges (Area ~ 0.25 m^2). Plant metrics did not significantly differ by location within the plots so only average values are reported here.

We sampled interstitial ammonium, nitrate + nitrite, sulfate, and sulfide at 7 of the plots in May, July and October 2002 (the last growing season). This sampling was done at all 5 treatment plots in one of the middle blocks (block 5) and in central areas (not associated with any specific treatment) in blocks 1 and 10 to assess if there were any

treatment or site differences along the 4 km length of the bankside environment where the experiment was conducted. Water samples were collected by installing plastic PVC tubes (30.48 cm height x 5.72 cm diameter) into the marsh near the edge of the treatment plots in block 5 in order to avoid disturbing the vegetation growing within the plots. The tubes were capped at both ends and had import ports at a depth of 15 cm, which is where the majority of *Spartina* roots are located (Howes et al. 1981, DeLaune et al. 1983). Water was sampled from the tubes after drawing water from the piezometer and allowing them to refill if possible. If water did not recharge within the well then the initial water was stored for use. These soils drained quickly and ample refill was difficult, so initial samples were often analyzed. Water was generally sampled during ebb tide.

Dissolved free sulfide was determined from water (10 ml) drawn directly from the tube, filtered through a 0.2 µm Gelman Acrodisc filter, and fixed in separate collection bottles containing 0.05 M zinc acetate (5 ml) for colorimetric analysis (Cline 1969). The remainder of the sample was filtered (pre-combusted Whatman GF/F, 47 µm filter) and samples for sulfate analysis underwent a second filtering through a 0.2 µm Gelman Acrodisc filter and were acidified with 100 µL of 70 % nitric acid. Samples were divided into aliquots. Ammonium samples were stored frozen whereas sulfate and sulfide samples were refrigerated prior to analyses. Ammonium concentration was analyzed colorimetrically and measured with a spectrophotometer (Koroleff 1983). Sulfate and sulfide were analyzed by M. Erickson and B. Porubsky in M. Joye's laboratory at the University of Georgia. Sulfate was analyzed by ion chromatography (EPA Method 300.1, Dionex LC20) and sulfide was measured spectrophotometrically (Cline 1969, Shimadzu UV-1601).

Pore water salinity was measured in the field in June and September 2001 and March and October 2002 with a portable refractometer (Leica Model 10419, automatically temperature compensated). Salinities did not significantly differ among treatments and thus reported salinities are the average of all samples taken during each sample period.

Data were analyzed using analysis of variance (ANOVA) with species and treatment as fixed effects (ANOVA; SAS Institute 2000, Sokal and Rohlf 1995). Initial measures were used in all cases as a standard covariate. A significance level (alpha) of 0.05 was used for all statistical analyses unless otherwise stated. All data were tested for normality (Shapiro-Wilk test statistic) and homogeneity of variance (Bartlett test statistic) prior to analysis. Where necessary, data were appropriately transformed to meet these assumptions. Tukey's post-hoc tests were used to look at the effect of treatment on plant performance. T-tests were used to test for differences between species by treatment (within each year) as well as to test for differences between years by treatment (within species).

RESULTS

Spartina Distribution along a Salinity Gradient

The density of both *S. alterniflora* and *S. cynosuroides* varied greatly along the length of the Altamaha River estuary during both 2000 and 2002 (Figure 4.3). *Spartina cynosuroides* densities peaked at approximately 76 shoots/m^2, whereas *S. alterniflora* densities reached as high as 300 shoots/m^2. In areas where these species overlapped, densities were comparable. While the data are highly variable, likely as a result of

habitat heterogeneity, *S. alterniflora* density generally decreased whereas *S. cynosuroides* density increased with increasing distance from the mouth. This distribution is in keeping with our expectation that *S. alterniflora* would dominate areas closer to the mouth of the river where salinities are higher and *S. cynosuroides* would be found in brackish areas. There were, however, differences between the two surveys. In 2000, *S. alterniflora* abundance was reduced upstream of 7 km, which is the point where *S. cynosuroides* became dominant. In 2002, this shift did not occur until approximately 10 km upriver.

In order to determine whether this upstream shift in the distribution of *S. alterniflora* was a consequence of changing salinity, we used data from two long-term projects (GCE-LTER and Georgia Rivers Land Margin Ecosystems Research [LMER] projects) to estimate average high tide surface water salinities for the two year period prior to our observations. High tide surface salinities were used based on observations that plant distributions in the Altamaha and Satilla Rivers in Georgia corresponded better with high-water salinity as opposed to average water column salinity measurements (Higinbotham et al. 2004). We averaged salinities over a two year period in order to integrate the conditions that plants were likely to respond to. Previous research investigating nutrient controls on *S. alterniflora* growth and distribution reported changes in plant growth parameters (such as aboveground biomass and height) after one or two growing seasons (Valiela and Teal 1974, Levine et al. 1998). Zedler and Beare (1986) reported that *S. foliosa* expanded in 1 year in response to changes in freshwater input. For the 2000 survey, we used salinity observations from 3 LMER cruises conducted in 1999 and 2000 (n=5 stations from 4-19 km). For the 2002 survey, we used salinity

observations from 6 LTER cruises conducted in 2001 and 2002 (n=12 stations from 4-22 km). In both cases logistic regressions between distance and salinity were used to estimate salinities at specific locations (J. Sheldon, pers. com.) Salinities were higher in 2001-2002 as compared to those in 1999-2000 (Figure 4.4).

In both surveys, *S. alterniflora* densities greatly increased where high tide salinities were greater than 14 psu, whereas at this same salinity, *S. cynosuroides* densities decreased (Figure 4.5). This distribution is consistent with the observations of Higinbotham et al. (2004) that the border between brackish and salt marshes along the Altamaha River occurred where high tide salinities averaged 15 psu. Moreover, Schubauer and Hopkinson (1984) reported that interstitial salinities in a *S. cynosuroides* stand, located on the Altamaha River, ranged from 2-14 psu over the course of the year. Thus, it may be that maximum salinities of 14-15 psu represent the physiological limit of *S. cynosuroides*.

In order to better evaluate both the differences between *Spartina* species and differences in plant characteristics in relation to salinity, plant measures from both 2000 and 2002 were averaged together and divided into either a lower (<14 psu) or higher (>14 psu) salinity range category and analyzed using a 2-way ANOVA with species and salinity as fixed effects. When compared in this way, there were significant differences between species in terms of height, tiller diameter, number of leaves, leaf area, number of shoots per m^2, % N and C:N ratios (Table 4.1). There were also significant differences between the lower (<14 psu) and higher (>14 psu) salinity ranges (ie. height, tiller diameter, leaf area, and % C). The only plant metric that showed a significant interaction between species and salinity range was plant height.

When the data was divided by species, differences within a species at low versus high salinities and differences between species within the low or high salinity ranges were identified as well as differences in growth between species within each salinity range (Table 4.2). Statistical significance for these comparisons was evaluated using the Bonferonni adjustment, which reduces the p-value for significance at the 95 % level to 0.001. When evaluated by salinity range, *S. alterniflora* had significantly more leaves above 14 psu, although several other performance characteristics were actually better at low salinities (i.e. height, tiller diameter, and photosynthetic area). In contrast, *S. cynosuroides* height, number of leaves, and tiller diameter were significantly greater below 14 psu. There were no significant differences in % N or % S tissue content or nutrient ratios (C:N or N:S) between high and low salinities for either species, but *S. cynosuroides* % C was higher at lower salinities. Comparisons between the two species at salinities less than or greater than 14 psu show that at high salinities (>14 psu), *S. alterniflora* had fewer leaves and smaller tiller diameters than *S. cynosuroides*, but at lower salinities (<14 psu) *S. alterniflora* was taller with greater tiller diameters, % N and % S. *S. alterniflora* plant's also had reduced % C and C:N ratios as compared to *S. cynosuroides* at salinities below 14 psu.

Growth and Recruitment of S. alterniflora and S. cynosuroides in Naturally Mixed Spartina Stands

In naturally mixed *Spartina* stands, *S. alterniflora* increased in density in all treatments over the course of the experiment (June 2001-October 2002; Table 4.3, Figure 4.6). This was true in mixed stands where *S. alterniflora* was initially removed (31.3 ± 4.8 plants/m^2 in June 2000 as compared to 117 ± 25 in October 2002; $p<0.0024$) and in

control stands where both species were present but neither manipulated (22.7 ± 2.9 plants/m^2 in June 2000 versus 63.1 ± 17.8 in October 2002; $p<0.0237$). *Spartina alterniflora* densities began to increase during the 2001 growing season and continued through 2002 as is evident from its increasing percent cover during mid-point sampling periods (September 2001 and March 2002; Figure 4.6). It should be noted that in treatment 2, where *S. alterniflora* plants were removed every 2 months from mixed *Spartina* stands, *S. alterniflora* densities were consistently higher than *S. cynosuroides* at all mid-point sampling points, indicating that it repeatedly out competed *S. cynosuroides* for available space. In addition to *S. alterniflora*'s invasion of mixed *Spartina* stands (where it was regularly removed), it also increased its density in monotypic stands where half the *S. alterniflora* was thinned (only at the start of the experiment) and had invaded similarly treated monotypic stands of *S. cynosuroides*.

Manipulating *S. alterniflora* or *S. cynosuroides* presence in mixed stands did not result in significant changes in the characteristics of either species (Table 4.4). No differences in *S. alterniflora* plant morphology or tissue nutrient concentrations were observed regardless of whether *S. cynosuroides* was removed from or present in mixed stands and regardless of whether *S. alterniflora* was thinned in monotypic stands. *Spartina cynosuroides* only had one instance where the manipulation of *Spartina* presence influenced growth: final aboveground biomass was much greater in monotypic stands where *S. cynosuroides* was thinned in comparison to either mixed stand condition. It may be that this species was able to grow better with less intraspecific competition than was present in other treatment plots, or it may be that there were microhabitat conditions

that were more favorable for *S. cynosuroides* growth in monotypic plots as compared to conditions in mixed stands.

Interstitial nutrients did not vary by treatment or location along the bankside, so values from all sites were averaged together for each sampling period (Table 4.5). Ammonium, sulfate and salinity values were within ranges of reported values for oligohaline marshes (Ewing et al. 1997, Howard and Mendelssohn 2000, Stribling and Cornwell 2001). Salinities did not significantly change over the entire 2 year period when all four measurements were compared ($p=0.0839$), however, significant increases in salinity were observed when only spring 2000 and fall 2002 were compared ($p=0.0452$) and when fall 2000 was compared with fall 2002 ($p=0.0271$). In contrast, sulfate concentrations decreased over the final year of the experiment ($p=0.0141$). Ammonium concentrations did not show any significant differences among seasons sampled.

The largest interstitial nutrient changes were seen in sulfide concentrations, which increased by two orders of magnitude, from 4.6 ± 0.6 μM to 572 ± 144 μM, between spring and fall 2002. The low sulfide concentrations in Spring 2002 likely reflect bankside flushing by tidal action and the oxidized status of the sediments at this point. The increasing sulfide values over the course of the growing season are within range of literature values reported for sites with lower water flows (King et al. 1982), but they may signify a more reduced environment. These sulfide concentrations are not high enough to hinder *S. alterniflora* growth: Bradley and Dunn (1989) found that sulfide levels below 1.5 mM did not inhibit tall form *S. alterniflora* growth. However, they reported that *S. cynosuroides* growth was limited at concentrations of 40 μM. This

suggests that *S. cynosuroides* growth was likely inhibited during the second year of the experiment, which may also have made it less able to colonize available space.

DISCUSSION

The observations reported here suggest that the estuarine distribution of *S. cynosuroides* is limited to average high tide water column salinities below 14-15 psu, and that this boundary shifted upstream in response to drought. During the same time period, we also found that *S. alterniflora* densities increased in areas that were previously mixed *Spartina* communities. Below, we further evaluate *S. cynosuroides* and *S. alterniflora* distributions with regard to changing salinities along the estuary. We then explore the possibility of using these observations for coastal management efforts.

Spartina alterniflora and S. cynosuroides Distribution along the Altamaha River Estuary

Vegetation surveys in both 2000 and 2002 showed great variability in plant density along the length of the Altamaha River estuary (Figure 4.3). However, when plotted against the average high tide salinity that the plants were likely to have experienced during the two years prior to each survey there was a natural breakpoint at approximately 14-15 psu in both years above which *S. alterniflora* densities greatly increased and *S. cynosuroides* sharply diminished. It is possible that this salinity range represents a physiological limit for *S. cynosuroides* success as most plant metrics were significantly reduced at salinities greater than 14 psu (Table 4.2). Schubauer and Hopkinson (1984) reported that interstitial salinities in *S. cynosuroides* stands located on the Altamaha River ranged from 2-14 psu. There is an early report that *S. cynosuroides*

communities could be found at salinities as high as 20 psu (Penfound and Hathaway 1938) but there have not been many investigations on this topic.

A potential downstream salinity boundary for *S. cynosuroides* growth and distribution is also supported by the results of the mixed species removal experiment described here as well as field reciprocal transplant experiments discussed in Chapter 1. The removal plots were located between 8 and 12 km from the mouth, and over the course of the experiment high tide salinities in this area increased from an average of 6.4 psu during pre-drought conditions (between 1994 and 1999) to 15.3 psu in 2001 and 11.3 psu in 2002 (high tide salinity averaged 13.4 psu during the two years [2001-2002] of the experiment; Table 4.6). Although the number of *S. cynosuroides* shoots in natural mixed stands (control treatment) remained relatively constant, its percent cover decreased from 46 % in 2001 to 22 % by the end of the experiment in 2002. This implies that *S. alterniflora* was much more successful in expanding in these plots, but that those *S. cynosuroides* that were already established remained. Reciprocal transplant experiments along the Altamaha River estuary showed that *S. cynosuroides* transplants survived but grew poorly in a salt marsh environment where interstitial salinities ranged from lower values during springtime (4-13 psu) to higher values during the fall (20-21 psu). In contrast, *S. alterniflora* transplants thrived in its home environment (Chapter 2).

The location of the 14 psu breakpoint shifted from approximately 6 km in 2000 (which is consistent with the location of the border observed by Higginbotham et al. [in press]) to 8.5 km in 2002. This upstream shift is important to note as we discuss the impact of drought conditions on *Spartina* distribution in more detail below.

Evaluating S. cynosuroides Estuarine Distribution as a Potential Bioindicator of Salinity Changes

The drought that affected Georgia between 1999 and 2002 provided us with the opportunity to document how vegetation communities along the length of the Altamaha River estuary might respond to changes in freshwater inflow. This type of distribution data, in conjunction with an understanding of the factors that cause these distributions, can help us predict how vegetative communities might respond to future perturbations. This type of information is critical, particularly in the context of potential future changes in salinity in response to both water resource policy and climate variation.

Increases in surface water withdrawals are anticipated in Georgia as groundwater sources diminish and coastal populations continue to grow. Coastal Georgia is dependent on groundwater from the Upper Floridan Aquifer and this resource is becoming contaminated via salt water intrusion in Brunswick, Savannah and Hilton Head Island (Clarke et al. 1990). As a result, the Georgia Environmental Protection Divison (GaEPD) imposed upper limits on groundwater use in several coastal counties in 1997. The GaEPD also set county goals for reducing groundwater withdrawals in an effort to minimize further damage to the aquifer (EPD 1997). At the same time, Georgia's population is increasing. It is estimated that between 1995 and 2015, Georgia's coastal counties will increase between 10 and 47 % (Turner 1999). Future development along the coast (as well as in the larger metropolitan centers further upstream such as in Atlanta) will likely be coupled to increased surface water withdrawals from the Altamaha River.

Climate variation can also affect freshwater inflow to estuaries. This can result from drought conditions, such as observed here, from increases in sea level (Stevenson et al. 1986, Day et al. 1993, Warren and Niering 1993), or from coastal land subsidence which can be either a natural process or the result of human development (i.e. levee and canal construction). All of these processes could potentially lead to increased upstream encroachment of salt water (DeLaune et al. 1983, Stevenson et al. 1986, Pezeshki et al. 1987, Warren and Niering 1993).

Decreased freshwater inflow, and the accompanying increase in salinity, can have great impacts on estuarine communities (Longley 1994, Sklar and Browder 1998, Alber 2002). Reduced inflows can result in changes in water quality (i.e. temperature, nutrient concentrations, turbidity, dissolved gases, and mineral concentrations) that negatively impact coastal ecosystems and organisms downstream (Sklar and Browder 1998, Wortmann et al. 1998). Sklar and Browder (1998) point out that the diversity and productivity of macrophytes, seagrasses, algae, and animals in coastal waters are determined by salinity tolerances, nutrient availability, and light regimes. These parameters are sensitive to the timing and amount of flow coming into coastal habitats. For example, Schuyler et al. (1993) linked the upstream movement of *Spartina* species in the Chesapeake Bay to long-term increases in salinity and Drinkwater and Frank (1994) reported changes in species composition, distribution, abundance, and health of fish and invertebrates as a result of decreased inflows. Visser et al. (2002) found that changes in vegetation type toward a community of more brackish species between 1997 and 2000 in a Louisiana estuary were associated with an increase in surface water salinity documented over the previous 5 years.

A necessary component for assessing whether there are adequate freshwater inflows into estuarine environments is to link biological indicators with actual salinity levels along an estuarine gradient (Alber 2002). Research in the Suwanee River estuary in Florida successfully identified a relationship between the relative abundances of freshwater (*Cladium jamaicense*) and salt tolerant (*Juncus roemarianus*) vegetation and the maximum salinity that these plants experienced in the field (Clewell et al. 1999). We were interested in determining whether we could develop a similar type of relationship in the distribution of *S. alterniflora* and *S. cynosuroides* along the Altamaha River estuary, as this would be a useful benchmark for coastal managers to assess how manipulating riverine inflows could influence plant community composition. We used data from both the 2000 and 2002 vegetation surveys and evaluated the relationship between % *S. cynosuroides* cover (as a proportion of the total density of both *Spartina* species) and either distance from the mouth of the estuary or average high tide salinity (Figure 4.7). There was considerable scatter in these plots but the data were best fit using logistic curves. There was little difference in either the shape or the location of the curve fit used to describe % *S. cynosuroides* in either survey year when the data were plotted against average high tide salinity, and both curves explained similar amounts of variability (r^2 for 1999-2000 was 0.49 whereas r^2 for 2001-2002 was 0.52). This result suggests that both *Spartina* species are responding similarly in both survey years to estuarine salinities. When the data were plotted against distance from the mouth of the estuary, the shape of the curve fits were again similar, but there was a clear shift in location between years.

If we evaluate these curves by considering where along the length of the estuary *Spartina* percent cover was 50 % *S. cynosuroides* and 50 % *S. alterniflora*, we find that

this location shifts approximately 3 km upriver between 2000 and 2002, from approximately 9.5 km to 12.4 km (Figure 4.7). Average high tide salinities at these locations were similar, approximately 8.9 psu and 9.9 psu respectively (average = 9.4 psu), so again the plants seem to be responding to salinity. Given this observation, we used a logistic equation developed for 6 LMER cruises between 1994 and 1999 and calculated that a salinity of 9.4 psu would be located approximately 8.4 km upstream during average flow conditions. Hence, we might expect to see 50 % *S. cynosuroides* cover at this location, whereas in 2000 it was approximately 40 % and by 2002 *S. cynosuroides* cover had dropped to 20 % (Figure 4.7).

The changes in percent cover observed here suggest that the *Spartina* community is responding to salinity changes fairly rapidly (within 2 years) and that these salinity changes can result in quite extensive shifts (~ 3 km) in distribution. Similar rapid shifts in marsh plant communities were observed by Zedler and Beare (1986) who reported that *S. foliosa* expanded in the San Diego River marsh system during 1980, 1982, and 1983 following periods of above-average rainfall and streamflow events. This documented increase in *S. foliosa* densities from 1980-1983 was reversed in 1984 when drought conditions increased channel salinities to 70 psu.

Responses in Mixed Spartina Stands

Under average conditions, 50 % *S. cynosuroides* cover occurs at approximately 8.4 km, but over the course of the drought, the location where there was 50 % *S. cynosuroides* cover moved upriver to 12.4 km. Given that the mixed experiment was established between 8 and 12 km, this offered us an ideal opportunity to explore how mixed *Spartina* communities respond to changing abiotic conditions. The expansion of

S. alterniflora (even after repeated removals from mixed *Spartina* stands) was evident in all treatment plots by the end of the first growing season (September 2001; Figure 4.6, Table 4.3) and by the end of the experiment (October 2002) it dominated all treatments except in monotypic stands of *S. cynosuroides* (where half of the plants were initially removed). However, even in these monotypic stands, where *S. cynosuroides* had been thinned, *S. alterniflora* invaded the available space over the course of the experimental period (Figure 4.6). It should be noted that while *S. alterniflora* densities increased over time, *S. cynosuroides* densities remained the same, suggesting that conditions were conducive for *S. alterniflora* expansion rather than *S. cynosuroides* reduction.

It is possible that salinity might not be the sole abiotic factor that enabled *S. alterniflora* to thrive in this experiment. Interstitial sulfide concentrations were significantly greater by the end of the second growing season as compared to initial observations (Table 4.5). This increase in sulfide concentration over the course of the growing season may have inhibited *S. cynosuroides*' ability to invade available space, as previous research suggests that sulfide concentrations greater than 40 μM inhibit this species' growth (Bradley and Dunn 1989). High sulfide concentrations were also observed where *S. cynosuroides* reciprocal transplants were planted in salt marsh environments. These transplants performed poorly in this environment, although the average sulfide concentration there (293 μM; Chapter 2) was considerably less than that observed here (572 μM).

Spartina species growth and performance were not influenced by neighbor presence or absence over the course of this experiment (Table 4.4). The fact that *S. alterniflora* invaded all treatments (including controls) suggests that the change in

salinity preempted the biotic interactions that might play a part in structuring this community. The dominance of abiotic controls over biotic interactions was also observed in reciprocal transplant experiments (Chapter 2) where *S. cynosuroides* survival and growth was poor in the salt marsh regardless of the presence or absence of neighbors.

Spartina alterniflora may have been more effective at expanding into available space in comparison to *S. cynosuroides* due to the possibility of differential salinity tolerance at an early life history stage. Specifically, since spring soil salinities were higher than average in this marsh (Tables 4.6 and 4.7) it is reasonable to expect that *S. alterniflora*, a salt marsh species, would have a greater tolerance than *S. cynosuroides*, a brackish marsh species, in early growth stages. A comparison between *S. alterniflora* and *S. cynosuroides* salinity tolerances in early growth stages has not, to our knowledge, been done. Rhizomatous growth is the most likely manner in which *S. alterniflora* and *S. cynosuroides* would expand their cover in this marsh habitat, as typical salt marsh environments have little viable seedbank reserves (Hopkins and Parker 1984, Hartman 1988, Odum 1988, Bertness and Shumway 1992, Shumway and Bertness 1992, Howard and Mendelssohn 2000). Shumway (1995) and Brewer and Bertness (1996) found that only species' that produced salt tolerant ramets (*Distichlis*) were able to successfully invade high salinity, patch environments. This could also be true for *S. alterniflora*. Successful early establishment can regulate plant distributions along environmental gradients via "temporal preemption" (Pickett and White 1985, Grace 1987, van der Valk 1992). Once established, *S. alterniflora* would be able to access available resources more readily than *S. cynosuroides*, thus leading to its expansion.

Zedler and Beare (1986) found that vegetation dynamics in the Tijuana Estuary, California were controlled by annual low salinity conditions that varied in duration and degree of salinity reduction. Salt marsh species established quickly in marshes when spring salinities were low but short in duration whereas brackish and freshwater species were able to establish and dominate if spring salinity conditions were low and more persistent. The "low salinity gap" found in California in the spring may have a counterpart in Georgia as the "high salinity gap", as springs where drought conditions are persistent may favor *S. alterniflora* establishment over *S. cynosuroides*.

Additionally, *S. alterniflora* displays a "guerilla" or runner rhizome morphology that allows it to quickly invade available space and establish itself rapidly before species with less advantageous rhizome morphologies (turf morphologies) can invade (i.e. *Juncus*, *S. patens*) (Bertness 1992, Brewer and Bertness 1996). This is unlikely to be a large advantage for *S. alterniflora* over *S. cynosuroides* as visual inspections show similar rhizome morphologies for these species. No comparisons between rates of vegetative growth of these two species under a range of environmental conditions have been conducted to determine if one species is "faster" than the other. It is possible that clonal integration between tillers outside of the treatment plots and tillers within plots could also enhance *S. alterniflora* expansion (Shumway 1995). Belowground assessment was not conducted. It may be that the increase in *S. alterniflora* within a plot was not only from tillers within the plot, but also from outside plants that were able to vegetatively expand into the plot. We were unable to quantify sources of re-growth within the plots. Again, little research comparing rates of resource allocation, or clonal integration, between these

two species has been completed to explicitly examine if one species has an advantage over the other in these areas.

Potential Recovery

It is not clear if the observed upstream shift in *S. alterniflora* and its rapid expansion into previously mixed *Spartina* environments is going to be permanent for the area and/or possibly continue to expand further upstream, or if the return of normal inflow will shift *S. cynosuroides* communities back down the river. Higinbotham et al. (2004) did not observe much change in brackish/freshwater marsh boundaries over the course of 40 years (using vegetation maps from 3 periods; 1953, 1974, 1993) suggesting that any previous upstream incursion of *S. alterniflora* did not persist. Pearlstine et al. (1993) analyzed the impacts of removing a tide gate on the Savannah River on vegetation communities. The presence of the tide gate had increased water and soil salinities 2-6 miles further upstream and this had resulted in the replacement of freshwater marsh with brackish plant species. They estimated that the freshwater marshes that were previously invaded by brackish marsh species (*Scirpus validus*), when the tide gate was operational, would increase in area by 340 % after gate removal. It is possible that *S. cynosuroides* may be able to out-compete *S. alterniflora* after the return of lower salinity conditions and this will be assessed with additional monitoring efforts along the Altamaha River estuary in Fall 2004.

Manipulative fieldwork by Howard and Mendelssohn (1999, 2000) found that marsh recovery or persistence of new community patterns resulting from disturbance was dependent upon species' tolerance to stress, the level of stress, and the duration of the stressful conditions (short-term pulses versus prolonged conditions). *Sagittaria*

americanus, a species with a greater salinity tolerance than *S. lancifolia*, persisted under high stress conditions. When stress conditions were alleviated, *S. lancifolia* had not recovered a month later (note: this experiment did not track vegetation response past one month). Flynn et al. (1995) also examined *S. lancifolia* recovery after a salinity stress and found some recovery after 10 months of low stress conditions. Baldwin and Mendelssohn (1998) found that there was a difference in recovery of marsh vegetation when the marsh disturbance was either lethal (above and belowground plant components killed) or non-lethal (aboveground components killed but belowground rhizomes still intact). Non-lethal disturbance patches were quickly reestablished by the previously dominant species (through resprouting) whereas lethal disturbances led to changes in community structure as seed regeneration and vegetative growth from outside the patch occurred. The spatial extent of the disturbance also has consequences for whether changes in marsh vegetation diversity are temporary or persistent over longer periods of time. Colonization of large disturbance patches with high soil salinities in New England marshes is often dominated by salt-tolerant species that facilitate colonization by competitively dominant species (Shumway and Bertness 1994). However, succession in smaller disturbance patches seems to be driven by competitive interactions between marsh dominants (i.e. *S. patens* and *Juncus*).

It is clear that the long-term (at least 2 year) high salinity conditions in this mixed site favored *S. alterniflora* vegetative establishment and growth to a greater extent than *S. cynosuroides*. However, if there was a sufficient period of low-salinity conditions, it is entirely possible that *S. cynosuroides* density would increase under less saline conditions. Drought conditions in Georgia have abated since 2003, resulting in increased freshwater

inflow and reduced salinities in the area of our study (between 8-12 km; Table 4.6).

The information that we gathered from the vegetation survey concerning *Spartina* distribution along the marsh and the results from the mixed species removal experiments suggests that *S. alterniflora* is increasing (or has the ability to increase) with increasing distance from the mouth of the estuary and that the point where marsh communities are half *S. alterniflora* and half *S. cynosuroides* can rapidly shift upriver under low flow, high salinity conditions. Further work has to be completed such that stronger linkages between reductions in freshwater inflows, changing salinity concentrations along the length of the estuary, and directional shifts in *Spartina* communities along the estuary can be established.

The establishment of reasonable costal management policies must be based on as much real-time field data as is possible. This work provides important baseline information on *S. alterniflora* and *S. cynosuroides* distribution and their response to increased salinity conditions along the Altamaha River estuary. It may be useful to track percent *Spartina* changes over time along the entire length of the estuary in order to determine whether estuarine conditions continue to change now that the drought is over. Continued ecological research that explores relationships between estuarine salinity and the distributions of plants and animal communities along the length of an estuary can offer policymakers benchmarks that can help describe how an estuary responds to environmental changes such as reduced freshwater inflows, rising sea levels and coastal land subsidence.

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Table 4.1. ANOVA table for plant parameters with species (*S. alterniflora* or *S. cynosuroides*), salinity range (<14 psu or >14 psu), and the interaction between species and salinity as the main effects in the model. Significant results ($p < 0.05$) are indicated in boldface (df=3).

Growth Characteristic	**Species**	**Salinity Range**	**Species X Salinity Range**
Height	**<0.0001**	**<0.0001**	**0.0088**
Tiller diameter	**<0.0001**	**<0.0001**	0.3624
No. Leaves	**0.0010**	0.1180	**<0.0001**
Leaf Area	**0.0021**	**0.0001**	0.5398
Biomass	0.1619	0.0531	**0.0170**
No. Seedheads/m^2	0.0717	0.4099	0.0616
No. Shoots/m^2	**0.0002**	0.2593	**0.0002**
% N	**<0.0001**	0.4970	0.1281
% C	0.9317	**0.0289**	**<0.0001**
% S	0.2105	0.4506	**0.0061**
C:N Ratio	**<0.0001**	0.7803	**0.0358**
N:S Ratio	0.4478	0.1774	**0.0038**

Table 4.2. *S. alterniflora* (SA) and *S. cynosuroides* (SC) plant metrics means ± SE (number of plants measured) for low (< 14 psu) and high (> 14 psu) salinity observations, regardless of survey year. Significant t-tests either within a species between salinity ranges (p-value column) or between species within a salinity range (p-values at bottom of each cell) are in boldface (df=1).

Metric	Low (<14 psu)	High (>14 psu)	p-value
Height			
SA	1.2 ± 0.03 (714)	0.99 ± 0.02 (611)	**<0.0001**
SC	1.6 ± 0.02 (1019)	1.2 ± 0.03 (63)	**<0.0001**
	0.0028	**0.0078**	
No. leaves			
SA	8.2 ± 0.2 (269)	9.3 ± 0.2 (185)	**0.0004**
SC	8.7 ± 0.2 (489)	6.5 ± 0.2 (39)	**0.0006**
	0.1952	**<0.0001**	
Tiller diameter			
SA	9.4 ± 0.1 (276)	8.1 ± 0.1 (185)	**<0.0001**
SC	8.6 ± 0.1 (483)	6.8 ± 0.3 (39)	**0.0009**
	0.0003	**0.0001**	
Leaf Area			
SA	719 ± 29 (253)	576 ± 29 (185)	**0.0007**
SC	609 ± 23 (454)	411 ± 32 (38)	**0.0149**
	0.0037	**0.0134**	
Biomass			
SA	180 ± 31 (24)	197± 25 (17)	0.6857
SC	217 ± 20 (39)	57.2 ± 18.4 (5)	**0.0081**
	0.2965	**0.0081**	
No. Shoots/m2			
SA	55.9 ± 10.9 (13)	141 ± 37 (6)	**0.0098**
SC	53.9 ± 6.3 (18)	6.3 ± 2.0 (4)	**0.0024**
	0.8650	**0.0197**	
No. Seedheads/ m2			
SA	1.7 ± 0.3 (25)	3.4 ± 0.8 (31)	0.0710
SC	1.8 ± 0.3 (38)	1.1 ± 0.1 (9)	0.2390
	0.9179	0.1225	
% N			
SA	1.4 ± 0.04 (37)	1.3 ± 0.04 (21)	0.1019
SC	1.0 ± 0.02 (48)	1.0 ± 0.05 (8)	0.5278
	<0.0001	**0.0076**	
% C			
SA	41.9 ± 0.2 (37)	42.9 ± 0.3 (21)	**0.0075**
SC	43.7 ± 0.2 (48)	41.1 ± 0.7 (8)	**0.0003**
	<0.0001	**0.0084**	
% S			
SA	0.37 ± 0.02 (37)	0.31 ± 0.03 (21)	0.0814
SC	0.24 ± 0.02 (48)	0.35 ± 0.03 (8)	0.1843
	<0.0001	0.4296	
C:N			
SA	37.0 ± 1.2 (37)	40.8 ± 1.9 (21)	0.0814
SC	52.2 ± 1.4 (48)	47.3 ± 3.8 (8)	**0.0214**
	<0.0001	0.0966	
N:S			
SA	9.4 ± 0.5 (37)	11.3 ±1.0 (21)	0.0802
SC	12 ± 0.9 (48)	7.0 ± 0.6 (8)	**0.0303**
	0.0275	**0.0173**	

Table 4.3. Number of *S. alterniflora* and *S. cynosuroides* shoots per m^2 at the beginning of both years of the experimental manipulation of naturally mixed *Spartina* stands (2001 and 2002) by treatment. N=10 unless otherwise noted. Differences between *S. alterniflora* and *S. cynosuroides* number of plants in each treatment in October 2002 are indicated by: * <0.05, ** <0.01. Different letters indicate significant differences among years by treatment within a species. Bold values in October 2002 indicate significant differences in density in either *S. alterniflora* or *S. cynosuroides* in comparison to June 2001.

		S. alterniflora				
		SA-sc	**SC-sa**	**Mx Stand**	**½ SA**	**½ SC**
2001	**June**	31.3 ± 4.8[a]	34.0 ± 3.3	22.7 ± 2.9	48.3 ± 5.1	4.6 ± 2.7
	September	95.9 ± 13.7 (9) [b]	43.0 ± 8.1	56.7 ± 16.1	70.6 ± 19.1	8.6 ± 3.6
2002	**March**	104 ± 11 (9) [b]	61.8 ± 14.7	64.7 ± 10.4	89.6 ± 16.8	8.3 ± 3.7
	October	**117 ± 25 (9)**** [b]	45.9 ± 12.3	**63.1 ± 17.8 (8)***	78.7 ± 15.9 (9)	13.3 ± 4.5 (6)

		S. cynosuroides				
		SA-sc	**SC-sa**	**Mx Stand**	**½ SA**	**½ SC**
2001	**June**	31.8 ± 4.5	34.2 ± 4.8	19.7 ± 2.4	1.7 ± 1.1	30.5 ± 3.0
	September	19.7 ± 13.9 (9)	28.9 ± 4.5	20.3 ± 4.6	20.1 ± 14.0	18.6 ± 4.8
2002	**March**	17.6 ± 6.0 (9)	32.1 ± 7.1	23.7 ± 7.2	3 ± 2.9	34.8 ± 7.1
	October	**2.1 ± 0.73 (9)**	26.8 ± 7.9	18.1 ± 4.2 (7)	26.3 ± 20 (4)	31.1 ± 7.5 (8)

Table 4.4. Final *S. alterniflora* (SA) and *S. cynosuroides* (SC) plant metrics (mean ± SE; N) in a naturally mixed *Spartina* stand. Treatments are mixed stands with either SA or SC removed (SA-sc or SC-sa respectively), control stands with both SA and SC present, and monotypic stands of SA and SC with ½ of the plants removed. Different letters indicate significant differences among treatments. F and p-values are from ANOVAs and indicate significant differences among treatments.

S. alterniflora

	Treatment				
Metric	SA – sc	SA with SC	½ SA	F-value	P-value
Height	58.4 ± 9.8 (8)	4.4 ± 0.5 (8)	4.6 ± 0.3 (9)	0.30	0.7445
No. Leaves	22.6 ± 2.6 (8)	21.3 ± 2.6 (8)	23.4 ± 2.2 (9)	0.50	0.6155
Tiller Diameter	371 ± 55 (8)	28.3 ± 1.6 (8)	25.4 ± 0.8 (9)	1.02	0.3786
Leaf Area	2048 ± 267 (8)	2034 ± 259 (8)	1833 ± 228 (9)	0.09	0.9177
Final Biomass	480 ± 104 (8)	423 ± 116 (8)	472 ± 92 (9)	0.36	0.6983
% N	1.3 ± 0.1 (3)	1.4 ± 0.2 (3)	1.3± 0.1 (6)	0.25	0.7850
% C	45.1 ± 2.7 (3)	43.3 ± 0.5 (3)	43.0 ± 0.7 (6)	0.69	0.5244
% S	0.54 ± 0.10 (3)	0.40 ± 0.08 (3)	0.33 ± 0.05 (6)	2.60	0.1284
C:N	38.3 ± 0.7(3)	36.4 ± 4.7 (3)	39.6 ± 3.3 (6)	0.19	0.8313
N:S	6.1 ± 0.7 (3)	8.3 ± 0.5 (3)	9.9 ± 1.3 (6)	2.21	0.1655

S. cynosuroides

	Treatment				
Metric	SC – sa	SC with SA	½ SC	F-value	P-value
Height	31.9 ± 8.1 (10)	5.6± 0.3; (7)	5.2 ± 0.6 (9)	3.08	0.0654
No. Leaves	19.4 ± 1.4 (10)	21.5 ± 2.3 (7)	21.6 ± 2.4 (9)	0.30	0.7461
Tiller Diameter	181 ± 44 (10)	29.1± 3.2(7)	26.4 ± 4.2 (9)	1.89	0.1732
Leaf Area	1485 ± 302 (10)	1685 ± 312 (7)	2293 ± 331 (9)	1.27	0.300
Final Biomass	276 ± 109 (10)[a]	269 ± 72 (7)[ab]	489 ± 79 (9)[b]	3.67	**0.0413**
% N	1.0 ± 0.02 (3)	0.82 ± 0.03 (3)	0.88 ± 0.06 (5)	2.35	0.1575
% C	44.3± 0.3 (3)	44.4 ± 0.3 (3)	43.3 ± 1.0 (5)	0.62	0.5597
% S	0.35 ± 0.04 (3)	0.37 ± 0.11 (3)	0.38 ± 0.05 (5)	0.08	0.9266
C:N	51.7± 0.9 (3)	63.5 ± 2.3 (3)	58.4 ± 5.1 (5)	1.40	0.3002
N:S	6.7 ± 0.6 (3)	6.4 ± 25.1 (9)	5.6 ± 0.8 (5)	0.24	0.7915

Table 4.5. Interstitial nutrient conditions in mixed species removal area (Mean ± SE; N). F and p-values are from ANOVAs and indicate significant differences among the sampling points.

Nutrient	**June 2001**	**September 2001**	**March 2002**	**July 2002**	**October 2002**	**F-value**	**p-value**
NH_4 (uM)	na	na	12.5 ± 8.7 (4)	49.2 ± 12.5 (6)	56.2 ± 30.7 (7)	0.80	0.4682
SO_4^{-2} (mM)	na	na	8.6± 0.7 (5)[a]	4.7 ± 1.7 (7)[ab]	2.8 ± 0.5 (7)[b]	5.62	**0.0141**
H_2S (uM)	na	na	4.6 ± 0.7 (3)[a]	108 ± 72 (7)[a]	572 ± 144 (6)[b]	7.23	**0.0077**
Salinity (psu)	10.3 ± 0.4 (45)	11.0 ± 0.3 (45)	10.6 ± 0.8 (9)	na	12.9 ± 0.4 (4)	2.38	0.0839

Table 4.6. Altamaha River average high tide salinities at the mixed *Spartina* experimental area (located between km 8 and 12). Salinities were calculated using logistic regressions based on data collected during GA-LMER and LTER monitoring cruises (J. Sheldon, pers. com). Salinity data reported for 1994-1999 and 1999-2000 are from GA-LMER cruises (6 cruises between 1994 and 1999, and 3 cruises over the period between 1999 and 2000). The remaining years of salinity data (2000-2003) represent data collected during LTER cruises (6 cruises during 2001-2002, 2 cruises during 2001, 4 cruises during 2002, and 3 cruises during 2003).

	Calculated Average High Tide Salinities					
Approximate km from mouth of estuary	**1994-1999**	**1999-2000**	**2001-2002**	**2001**	**2002**	**2003**
8	**9.46**	**10.31**	**16.42**	**18.32**	**14.48**	**3.10**
9	**7.84**	**9.33**	**15.03**	**16.99**	**12.96**	**2.65**
10	**6.27**	**8.30**	**13.51**	**15.50**	**11.35**	**2.22**
11	**4.95**	**7.35**	**12.05**	**14.03**	**9.85**	**1.85**
12	**3.37**	**6.04**	**9.94**	**11.87**	**7.77**	**1.39**

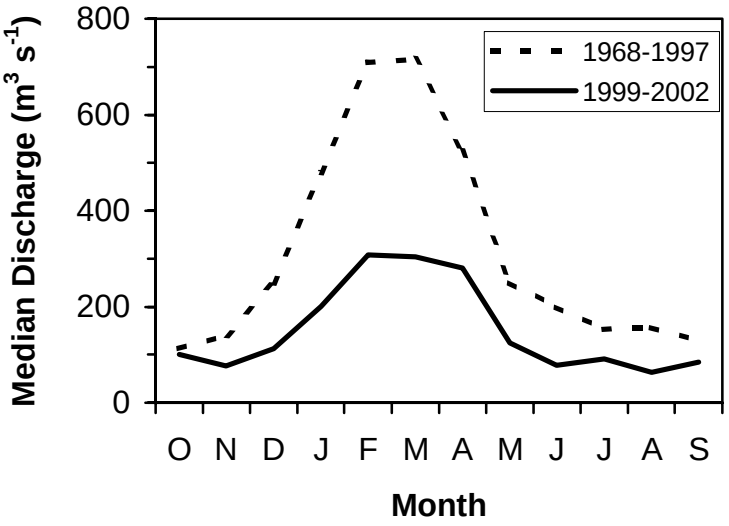

Figure 4.1. Monthly median Altamaha River freshwater inflow from 1968-1997 (dashed line) in comparison to drought years 1999-2002 (solid line) (Sources: Based on USGS data at Doctortown corrected for the ungauged portion of the watershed, and J. Sheldon, UGA).

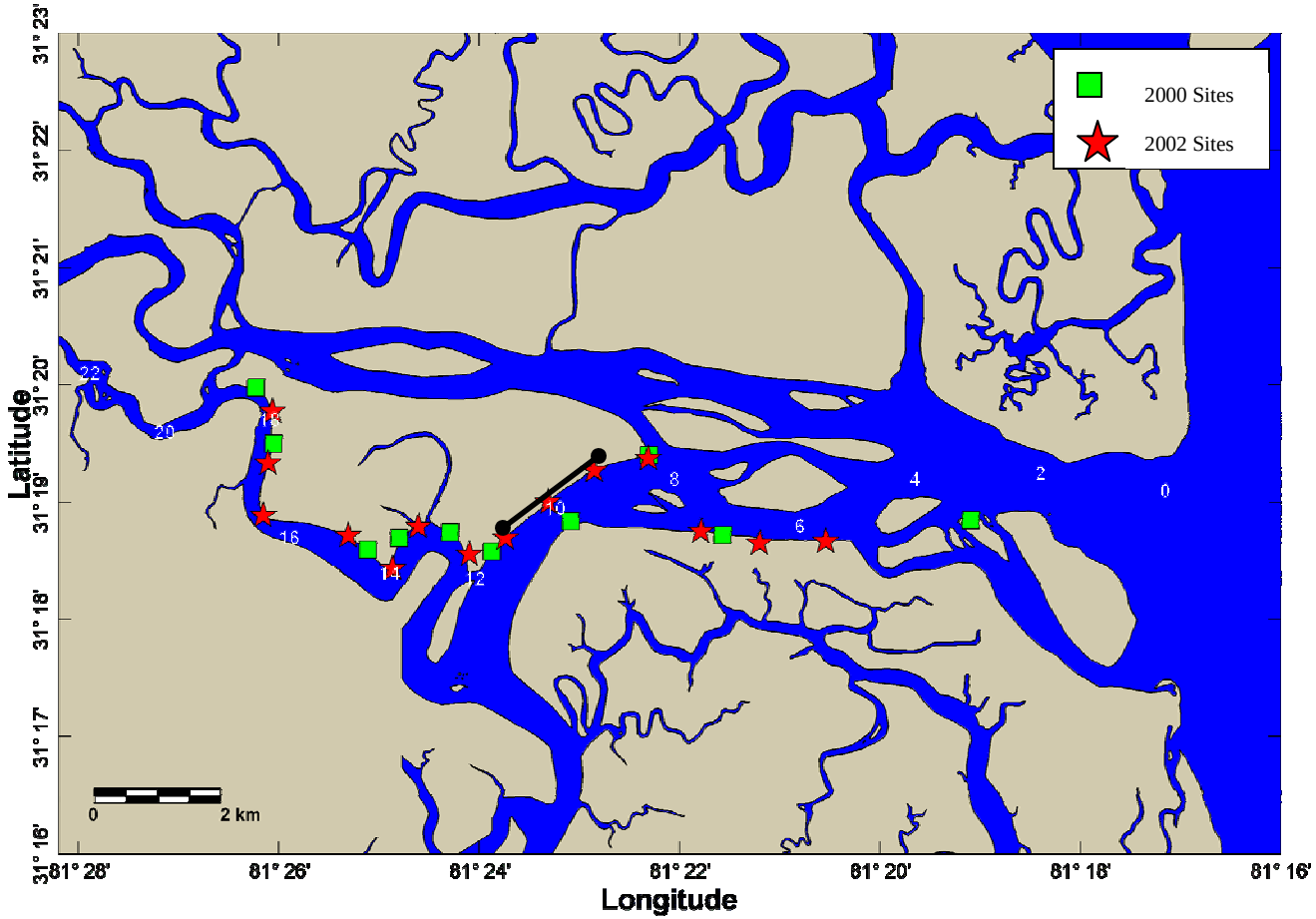

Figure 4.2. Altamaha River estuary showing vegetation survey sites in 2000 (squares) and 2002 (stars) as well as the mixed species removal sites (bracketed area between 8 and 12 km from the mouth).

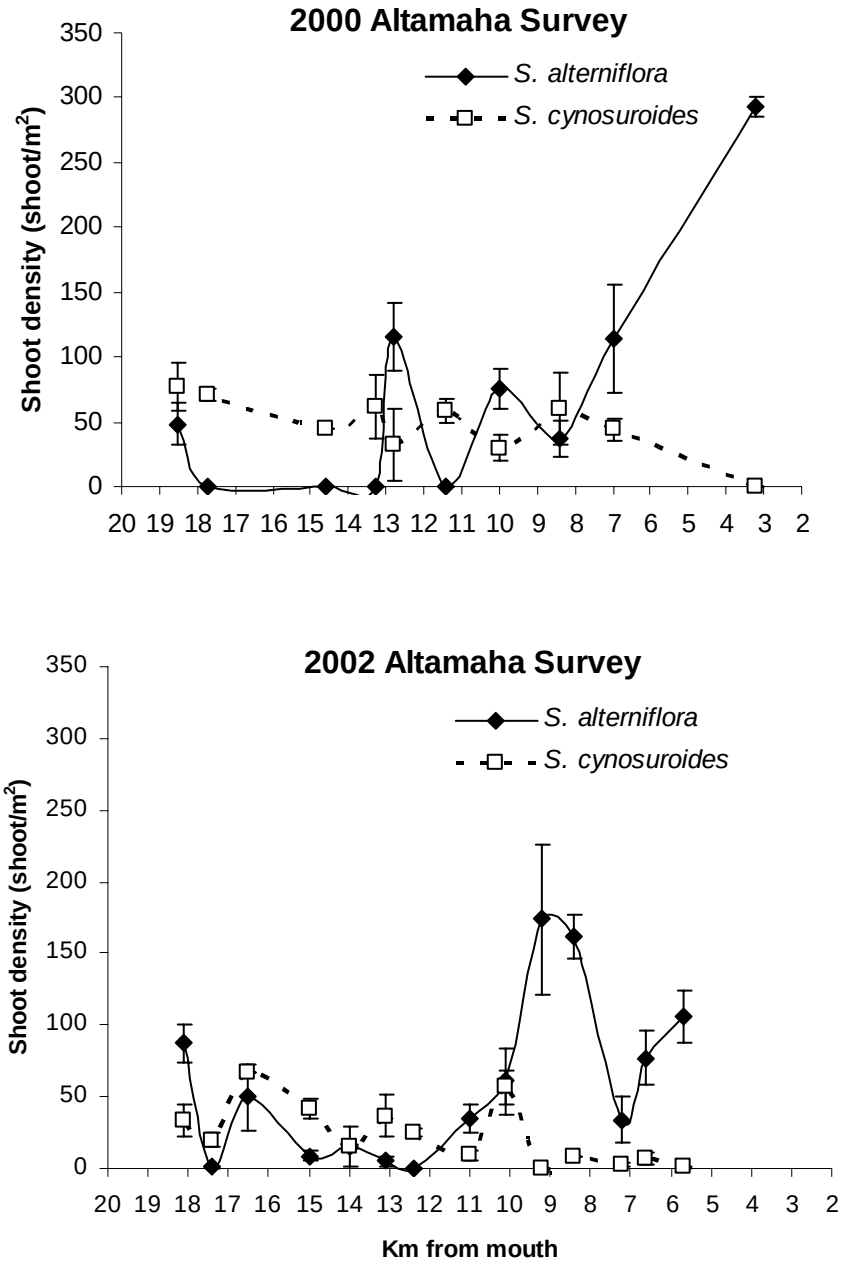

Figure 4.3. *S. alterniflora* (solid line) and *S. cynosuroides* (dashed line) densities (± S.E.)along the length of the Altamaha River estuary in both survey years (2000, 2002).

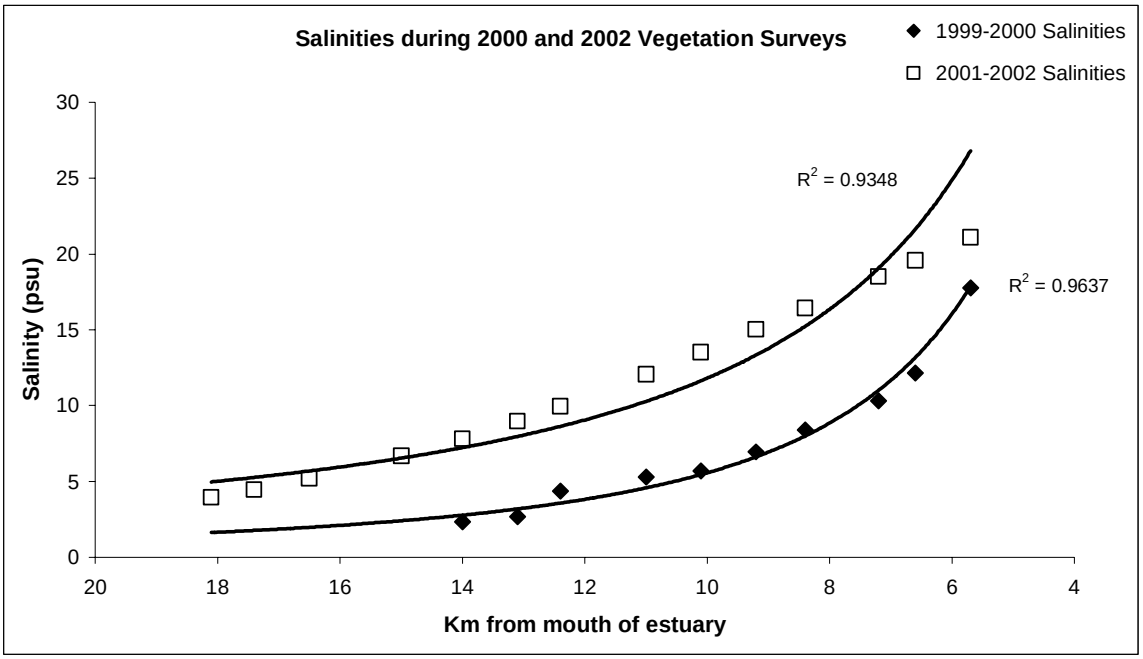

Figure 4.4. Average high tide surface water salinities observed in the Altamaha River during 1999-2000 (diamonds) and 2001-2002 (squares). Lines represent logistic curves. Data are from the Georgia Rivers LMER program (1999-2000) and the Georgia Coastal Ecosystem LTER project (2001-2002) (Source: J. Sheldon).

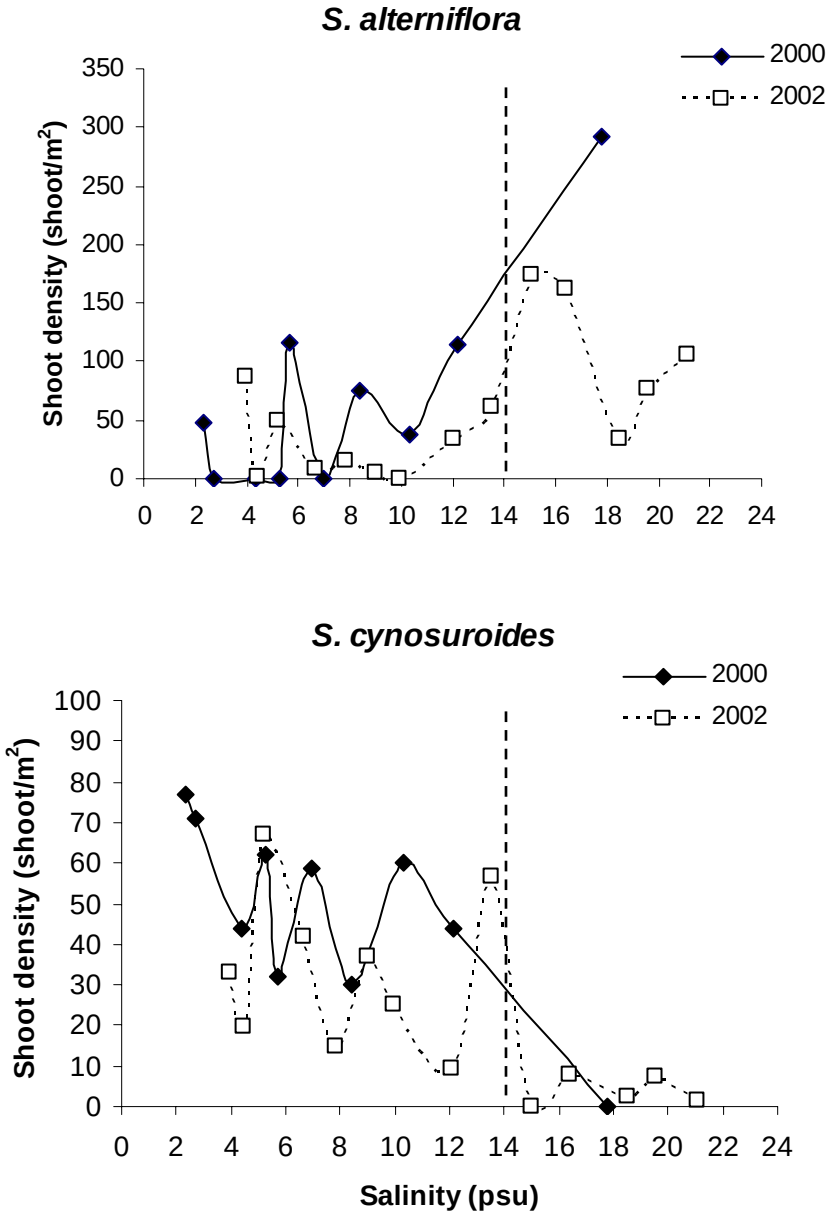

Figure 4.5. *S. alterniflora* and *S. cynosuroides* densities along the salinity gradient of the Altamaha River estuary during the 2000 (solid line) and 2002 (dashed line) surveys. Vertical dotted line indicates where 14 psu is located.

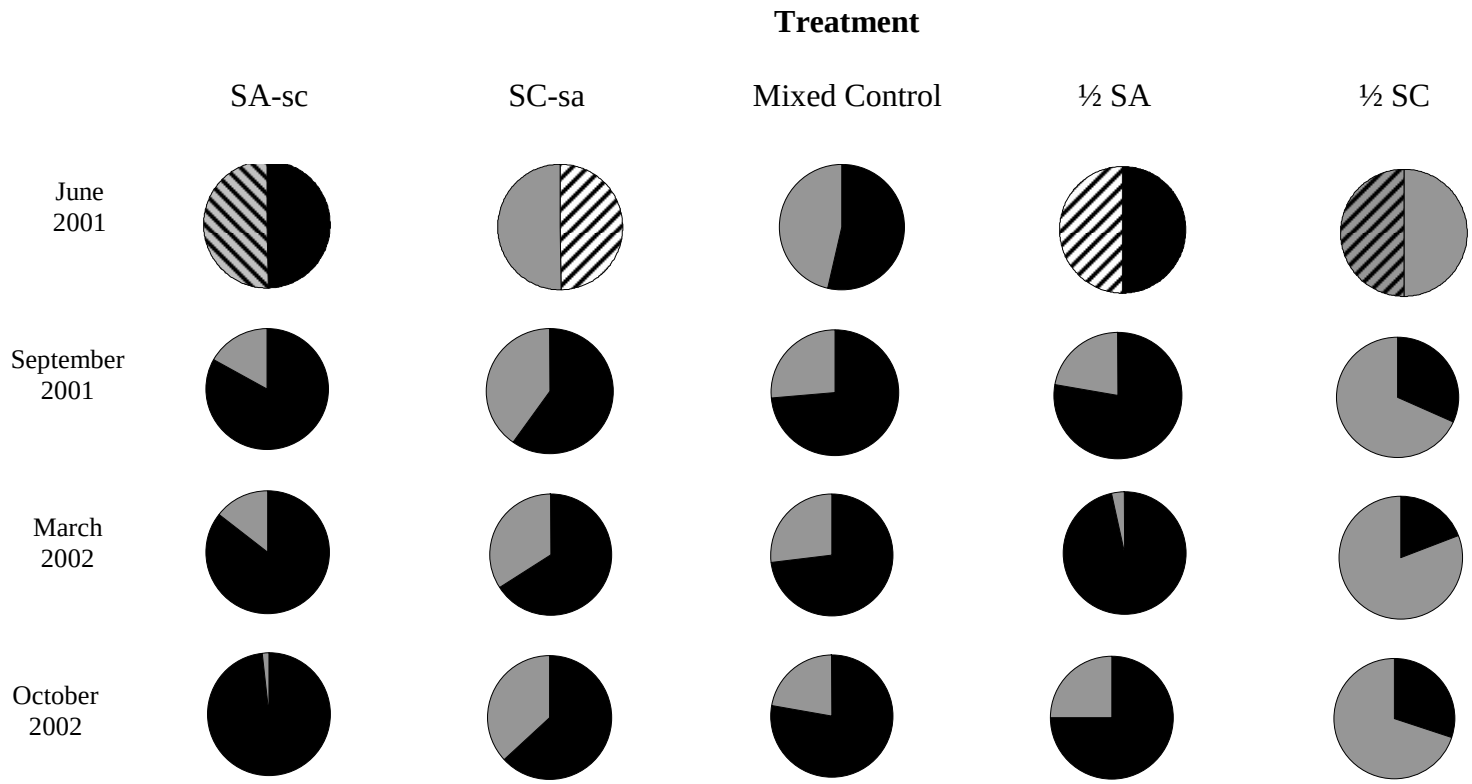

Figure 4.6. Mixed species removal experiment: *S. alterniflora* and *S. cynosuroides* percent coverage in each treatment over the course of two growing seasons. *S. alterniflora* is represented by black pie pieces whereas *S. cynosuroides* is represented by grey pie pieces. Hatched areas in June 2001 indicate where either *S. alterniflora* or *S. cynosuroides* were removed. Treatments are mixed stands of *Spartina* with either *S. cynosuroides* removed (SA-sc) or *S. alterniflora* removed (SC-sa), mixed *Spartina* control stands, and monotypic *S. alterniflora* and *S. cynosuroides* stands with half of that species removed (½ SA and ½ SC respectively). In order to maintain the appropriate treatments in mixed *Spartina* stands, removals of either *S. cynosuroides* or *S. alterniflora* from mixed stands of *Spartina* (i.e. SA-sc or SC-sa) occurred approximately every two months over the course of the experiment.

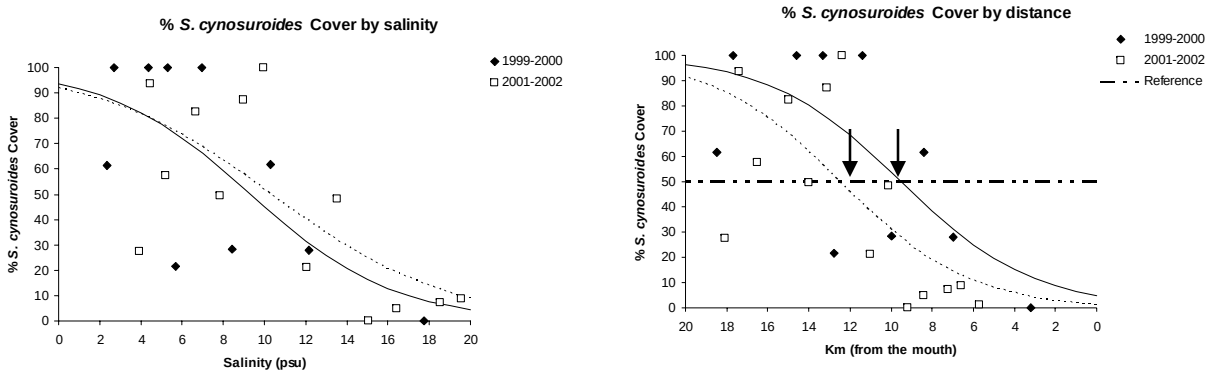

Figure 4.7. Percent *S. cynosuroides* cover along the length of the Altamaha River estuary in both survey years by salinity and distance. Reference line indicates where communities are 50 % dominated by *S. cynosuroides*. Non-linear regression lines were used to fit data from both survey years separately. The equation for each of the lines above was y=SWsal + (FWSal-SWSal)/(1 + exp((inflection – x)/scale)) where SWsal =100, FWSal =0 and the inflection and scale varies for each of the lines. The inflection values for lines corresponding to % *S. cynosuroides* cover by salinity were 9.3173 and 10.3195 whereas the scale values were 3.4780 and 4.2183 for 1999-2000 and 2001-2002 respectively. The r^2 values were 0.47 for 1999-2000 and 0.46 for 2001-2002. The inflection values for lines corresponding to % *S. cynosuroides* cover by distance were 9.5165 and 12.4232 whereas the scale values were -3.1979 and -3.1027 for 1999-2000 and 2001-2002 respectively. The r^2 values were 0.49 for 1999-2000 and 0.52 for 2001-2002. Arrows indicate the shift in community cover between 2000 and 2002 surveys (from ~9.5 km to ~12.4 km respectively).

CHAPTER 5

CONCLUSIONS

The main goal of this dissertation was to examine whether abiotic (salinity and sulfate) and biotic (plant-plant interactions) mechanisms could describe the distribution of *Spartina alterniflora* and *S. cynosuroides* along the length of the estuarine gradient of the Altamaha River, Georgia. I was also able to document shifts in the distribution of these species and evaluate the response of naturally mixed species' stands to an extended drought from 2000-2002. I conducted reciprocal transplant experiments and mixed species removals in the field and also carried out greenhouse experiments that investigated the response of these *Spartina* species to a variety of salinity, sulfate and neighbor treatments. The results of this research suggest that the lower estuarine distribution of *S. cynosuroides* is constrained by harsh abiotic conditions, most likely high salinity. There was some evidence to support the notion that the upper estuarine distribution of *S. alterniflora* may be constrained by a sulfate nutrient requirement that is not satisfied in brackish marshes, although this requires further investigation. Competition between these two *Spartina* species did not appear to have a strong role in either the distribution of these communities along the length of the estuary. Lastly, the increased salinities that occurred during the drought resulted in an upstream shift in the distribution of *S. alterniflora* communities along the Altamaha River, and an increase in *S. alterniflora* density in previously mixed *Spartina* stands.

The results of a reciprocal transplant experiment, described in Chapter 2, suggest that the mechanisms that control plant distribution in a single salt marsh (i.e. physiological tolerance and plant-plant interactions) are not entirely appropriate for describing *Spartina* distributions along the longitudinal estuarine gradient. *Spartina alterniflora* and *S. cynosuroides* transplants each survived and performed best in their natural habitat. *Spartina alterniflora* survival by the end of the experiment was 80 % in the salt marsh but only 20 % in the brackish marsh whereas *S. cynosuroides* survival in the brackish marsh was 60 % as compared to only 20 % in the salt marsh. The presence of conspecific neighbors in the salt marsh slightly reduced *S. alterniflora* plant performance whereas *S. cynosuroides* showed little response to the presence or absence of neighbors in either environment. These results support the theory that harsh abiotic conditions (possibly high salinity or sulfide conditions) constrain the distribution of *S. cynosuroides* downriver; however the poor survival of *S. alterniflora* in the brackish marsh regardless of the presence or absence of neighbors suggests that the upriver distribution of *S. alterniflora* is not maintained by competitive interactions.

Interstitial sulfate concentrations measured in both reciprocal transplant areas indicated that sulfate concentrations in the brackish marsh (0.84 ± 0.34 mM) were significantly less than those observed in the salt marsh (8.1 ± 0.8 mM). This low sulfate environment may have influenced the upper limit of *S. alterniflora* distributions as plant tissue analyses indicated that N:S ratios were greater in the brackish marsh as compared to the salt marsh. This greater N:S ratio was coupled to significant decreases in tissue % S in the brackish marsh. Additional macro- and micronutrient tissue concentrations (Al, Fe, K, Mg, Na, and Si) observed in *S. alterniflora* transplants were within range of

previously reported values and did not provide insight into controls on this species growth.

There are a number of research recommendations that could be followed to improve similar future reciprocal transplant experiments. Many of these involve modifying the sampling techniques utilized to assess environmental soil and water conditions. Interstitial water nutrient concentrations would be more detailed and accurate with the use of specially designed piezometers that sample over a series of depths in the marsh substrate. Water nutrient sampling would be enhanced with these piezometers as field anoxic conditions could be more easily maintained using Argon gas to purge the sampling wells, thus maintaining a sampling environment that enables a more accurate measurement of available interstitial sulfide and sulfate concentrations. The additional information that would be provided with a series of measurements over multiple depths would be helpful for better quantifying environmental growth conditions in the field. Additional soil conditions such as pH, Eh, soil porosity and organic matter content would also increase our understanding of the plant's growth environment. Ideally, interstitial nutrient sampling would occur on a more regular basis (multiple times over the course of a month) rather than on a quarterly basis in order to get a more comprehensive depiction of soil characteristics. Nutrient concentrations should also be processed more rapidly to avoid possible sample deterioration over time. The different microbial or mycorrhizal communities associated with either salt or brackish marsh soils were not assessed over the course of the experiment and these communities have the potential to influence transplant success. It may be that specific communities are associated with either salt or brackish marsh environments and when the microbial component is not transplanted into

a new transplant environment the survival and growth of the transplanted *Spartina* species is diminished. Further work exploring microbial communities in both environments could increase our understanding of what influences *Spartina* distributions.

In addition to knowing more about the soil and water conditions in the reciprocal transplant sites, a greater understanding of *Spartina* plant nutrient requirements would be useful for quantifying plant response. It is quite possible that plant nutrient analyses do not necessarily reflect the availability of specific nutrients in the soil, thus sediment nutrient analyses would be informative. There is considerable speculation that sulfur availability might control *S. alterniflora* distribution in the brackish marsh. However, little information is known concerning how much sulfur is contained in one of the osmolytes, dimethylsulphoniopropionate (DMSP), present in *S. alterniflora*. We are unaware of any research that has quantified how much tissue sulfur is lost (as DMSP or otherwise) during the drying and combustion process used to assess plant tissue nutrient concentrations. Thus, it may be that our reported sulfur values are underestimates of the actual sulfur content of either *S. alterniflora* or *S. cynosuroides* plant tissue (R. Kiene, personal communication). In addition to studies investigating the osmolyte DMSP, it would be useful to understand if *S. alterniflora* and *S. cynousoirdes* had different dominant osmolytes as this could potentially lead to differential selection pressures for one species over the other in either brackish or salt marsh habitats.

Lastly, *Spartina* transplant response to treatments might be better understood if additional steps were taken to ensure that the removal of neighbors was completely effective. It might be useful to increase the cleared radius around the transplants from 0.35 cm to a radius twice that size in order to reduce any shading or belowground

inhibition from plants located outside the transplant area. It might also be useful to quantify belowground biomass as a growth parameter in both the brackish and salt marsh, although this is typically time consuming and difficult to conduct with such similar *Spartina* species.

Further investigations of *Spartina* growth under manipulated salinity and sulfate conditions were conducted in a greenhouse environment in an effort to more clearly define distribution controls. The results from the greenhouse experiments, as reported in Chapter 3, supported the conclusions from the reciprocal transplant field experiment regarding the controls of *S. cynosuroides* lower estuarine distribution. In the first greenhouse experiment, *S. cynosuroides* survival was 100 % in all but one treatment (where it was 86 %) and in the second experiment this species had 100 % survival in five of six low salinity treatments and three of six high salinity treatments. These results supported the observations from field experiments where *S. cynosuroides* transplants performed better in lower salinity conditions than in high salinity environments. In contrast, greenhouse experiments with *S. alterniflora* showed the opposite results from field experiments. *Spartina alterniflora* survival and growth was best at lower salinities in the greenhouse but poorest at low salinities in the field. In the first greenhouse experiment, *S. alterniflora* survival was 100 % in four low salinity/low sulfate treatments but only 14 % in higher salinity/sulfate treatments and no plants survived in two of the highest salinity/sulfate treatments. *Spartina alterniflora* survival was better in the second experiment, but it still had significantly reduced survival in high salinity treatments, with up to 75 % mortality. It appeared that sulfate and neighbor treatments did not influence

S. alterniflora growth in the greenhouse, although full analyses of these effects was difficult given the high mortality.

Additional soil and plant tissue nutrient analyses were conducted to explore the possibility that *S. alterniflora* growth in the greenhouse was impacted by unusual nutrient availabilities (i.e. limiting nutrients or toxic levels of nutrients). It is possible that the poor *S. alterniflora* performance at higher sodium and sulfate concentrations was a result of the very large final ammonium concentrations measured in both experiments. High ammonium concentrations can be toxic for plant growth. However, both plants experienced the same porewater conditions so both plants should have been similarly inhibited at these high concentrations and this was not the case. *Spartina cynosuroides* survival was better in the treatments with high final ammonium concentrations as compared to *S. alterniflora*, although both *Spartina* species' growth parameters were similarly negatively affected in these treatments. Sulfate and nitrogen tissue concentrations were within range of previously reported values suggesting that these nutrients did not limit *S. alterniflora* growth. Results from additional elemental analyses suggest that iron limitation or zinc toxicity might be exacerbated in high salinity treatments and possibly have caused the reduced performance observed in these experiments.

There are several factors that could be further explored as a follow up to these experiments. First, it would be useful to identify the reason for the extremely high ammonium concentrations observed in these experiments. As part of this, it would be informative to compare concentrations in water sampled approximately 24 hours after treatments are delivered (rather than immediately) to better quantify what the plant is

experiencing. It may also be better to give the treatments daily rather than only three times per week, as this may reduce nutrient buildup over time. Soil pH was not measured over the course of the experiment and this type of information could be useful in understanding the abiotic conditions that might have led to higher ammonium concentrations in the pots. A mixture of sand and peat moss was used as the potting medium in these greenhouse experiments, and it is possible that the greater porosity of this mixture as compared natural marsh sediments had an unexpected negative impact on sediment/water chemistry and plant performance (i.e. possible superoxidation of sediments that the plants are not adapted for).

The unexpectedly poor *S. alterniflora* growth in both greenhouse experiments may have been a result of a number of physical parameters. Plant growth may have been inhibited by an inadequate pot size and so a larger pot (> 12 cm) might be used in the future to avoid the possibility of plants becoming root bound. There were a number of instances where rhizomes of both *Spartina* species were shooting out the bottom of the pots, suggesting that a larger pot could be a useful adaptation for future experiments. Larger pots would also increase the likelihood that neighbor effects would be observed as the plants would be able to grow larger and for a longer experimental period before the final harvest was necessary. Plant growth may have also been inhibited by the time of the year that the second greenhouse experiment was conducted, ideally follow-up experiments would be conducted over spring and summer months as opposed to fall months when plants may be senescing naturally (regardless of light and nutrient resources). There is also the possibility that the acclimation period during which time the plants salinity and sulfate treatments were increasing slowly to their final target

concentrations was not sufficient and inhibited plant growth and survival at an early stage. Longer periods of acclimation (> 3 weeks) might have improved *Spartina* plant survival.

The last chapter of this dissertation focused on changes in *Spartina* distributions along the Altamaha River estuary (Chapter 4). This chapter provided further insight into distributional controls of these two species under changing environmental conditions. Freshwater inflow to the Altamaha River decreased considerably over the years from 1999-2002 due to prolonged drought conditions. Consequently, saltwater penetrated further upstream such that salinities as large as ~10 psu were recorded 16 km from the mouth in 2001 and sustained average salinities of approximately 3 psu were observed 20 km upriver in 2000 and 2001. Under typical inflow conditions, salinities are consistently lower (<0.5 psu) in areas 20 km from the mouth of the estuary. Our hypothesis that *S. alterniflora* and *S. cynosuroides* would be spatially distributed along the estuary according to their salinity tolerances, with *S. cynosuroides* dominating in the low salinity habitat and *S. alterniflora* dominating at high salinities was supported from our vegetation survey results. When we analyzed where there was 50 % *S. cynosuroides* and 50 % *S. alterniflora* cover in 2000 versus 2002 we found that this demarcation shifted approximately 3 km upriver, from a location around 9.5 km from the mouth in 2000 to 12.4 km from the mouth in 2002. This suggests that *Spartina* communities can respond rapidly to increasing estuarine salinity conditions. The results from our mixed species removal experiment supported our survey observations, as *S. alterniflora* expanded and dominated in this area between 2001 and 2002, during which time this environment experienced high interstitial salinities. These results provide important baseline

information on *S. alterniflora* and *S. cynosuroides* distribution and their interactions under disturbance conditions along the Altamaha River estuary and may be used by policymakers as benchmarks that can help describe how an estuary responds to environmental changes such as reduced freshwater inflows, rising sea levels and coastal land subsidence.

In order to more fully understand how *Spartina* communities respond to changing environmental conditions along the Altamaha River estuary, an additional *Spartina* plant survey will be conducted in Fall 2004 as the severe drought conditions experienced between 1999-2002 have abated. Improvements for further research that focuses on where these two *Spartina* species co-exist would include increasing the size of the sampling quads (> 0.7 m) so that neighbor removal treatments might be more effective. It would also be informative to assess the belowground growth of these species under neighbor manipulation treatments, however, as stated previously, this type of effort is both time intensive and difficult given the similarity in the root design of these two species. It would be useful to have more detailed water and soil conditions in order to better understand any microhabitat differences within our experimental site. Improved sampling efforts, as described above with regard to the reciprocal transplant experiment, would also help us better define environmental conditions within the mixed *Spartina* species community.

Overall, the results of this dissertation suggest that *Spartina* distributions along an estuarine gradient are primarily controlled by abiotic conditions with biotic interactions playing a moderate, secondary role. The lower estuarine distribution limit of *S. cynosuroides* appears to be solely defined by abiotic parameters, namely physiological

tolerance to high salinity (or possibly sulfide). The upper estuarine distribution of *S. alterniflora* is not well understood, but seems to be primarily controlled by abiotic factors (possibly a sulfate requirement) rather than competitive interactions between species. Further experimentation is warranted to better understand whether *S. alterniflora* may have a sulfate requirement that is not met in these low salinity environments. Under reduced inflow conditions *S. alterniflora* and *S. cynosuroides* community's shift upriver, indicating that these species do respond to changing environmental conditions. More detailed investigations of how *Spartina* distributions along an estuarine gradient may change under disturbed conditions would be useful for both river and coastal management purposes.